

The V/A Ratio of the Smaller Vessels of the Bulbar Conjunctiva in Diabetes Mellitus

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In patients who have had diabetes mellitus for many years, more or less pronounced vascular abnormalities in one or more organs are very common. Clinically, these abnormalities usually appear as affections of the retina, kidney, heart and limbs. There are many reasons to assume that these long-term diabetic abnormalities are manifestations of a generalized diabetic vascular disease, a diabetic angiopathy.¹⁻⁴

Diabetic vascular disease of the retina stands apart from other vascular anomalies in diabetes because it can be directly observed and followed through its various phases by ophthalmoscopy. As early as 1890, diabetic retinopathy was a well-defined concept,⁵ and it has later been analyzed in numerous studies, of which it will suffice to mention the classic works of Waite and Beetham,⁶ Ballantyne⁷ and Hanum.^{7a}

Observations on retinopathy occupy a central position in the study of the nature of diabetic vascular disease, because this lesion is directly visible and because its ophthalmoscopic picture is characteristic. However, the special conditions which prevail in the eye due to the intra-ocular pressure make it difficult to draw conclusions about the general state of the blood vessels of the organism on the basis of observations made on the retinal vessels.

In the late 1940's, attention was directed to another vascular region, which may also be directly observed. In their studies of the so-called sludge phenomenon, Knisely et al.⁸ showed that the vessels of the bulbar conjunctiva are a suitable object of *in vitro* studies in man. In addition, the conjunctival vessels could be considered to be more representative of the general vascular status in the organism than those of the retina.

In a series of publications, J. Ditzel and his co-workers⁹⁻¹³ have reported studies of the conjunctival vessels in diabetes mellitus. The most important results of these investigations are the finding of angularity and tortuosity of the capillaries and venules, a low ratio

between the diameter of the arteriole and that of the corresponding venule (a/v ratio), fusiform dilatations of the venules, and perivascular edema and "hyaline" infiltrations. However, these changes in the conjunctival vessels were observed not only in long-term diabetics but also often in patients who had suffered from diabetes for only a few years.

With one exception, the vascular abnormalities observed in the conjunctivae of diabetics by Ditzel and his associates are difficult to assess quantitatively. The exception is the a/v ratio, which may be determined with a reasonable accuracy in standardized photographs. Ditzel and St. Clair¹⁰ developed a method for photographing the smaller vessels in the conjunctiva and described the determination of the a/v ratio in one patient. In the other publications, a/v ratios which had been estimated by direct microscopic observation are reported; these are given as 1:2, 1:3, 1:4, etc.

The following is a report of a comparative study of the ratio between the venules and the arterioles determined quantitatively in a series of patients with diabetes of shorter or longer duration and in a control nondiabetic group.

METHOD AND MATERIAL

Measurements of the diameters of the conjunctival arterioles and venules were performed by means of a measuring microscope, directly on the negatives of standardized photomicrographs of the conjunctiva taken through a microscope.

A Leitz stereobinocular microscope (Greenough type) with the tubes in the horizontal position, was used. A Contax I camera provided with a lens of the following specification: Tessar 1:2.8; F: 4.5 cm. was attached to one of the tubes of the microscope. A diaphragm 2.8 was used; exposure time 1/30 second.

Inspection of the conjunctiva and focusing on the area to be photographed were done through the other tube. The photographs were taken through a plane eyepiece 25 x and objective 4 x. The pilot light was a 12-volt incandescent electric lamp.

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The light source for the exposure was an electronic flash (Mecablitz 500) with the following specifications: light output 120 watt-seconds; color temperature 5,500° Kelvin; discharge time 1/750 second; current supply A.C., 220 volts.

The lighting setup used was a modification of the apparatus described by Bruzelius and Holm.¹⁴ By this arrangement, in which the beam direction of the pilot light and electronic flash are identical, it is possible to avoid disturbing reflections on the photographs by reflection-free adjustment before the exposures.

During the examination, the patient was seated on a chair facing the binocular microscope. His head was immobilized by an ophthalmic head rest supporting the chin and forehead. The light source was placed to the left of the patient's head. In all cases, photographs were taken of the temporal part of the left eye as it presented itself when the patient fixed his gaze on a target. The conjunctival area to be photographed was selected by means of an ocular micrometer so that it did not include the ciliary vessels at the limbus corneae, the exposed area being at least 5 mm. from the limbus.

Eight to ten exposures were made of the conjunctiva on Adox Docupan or Agfa document films. The measurements were made directly by means of an ordinary measuring microscope with an ocular 6 x and an objective lens 3 x. The film strip was placed in a special holder which fixed the negative during measurement and allowed passage of the strip to the next area to be measured. The measuring microscope was provided with a vernier giving an accuracy of 0.01 mm.

In the individual negatives, one or more *pairs of vessels* (arteriole and accompanying venule) were selected. Five measurements were made of the arteriole and venule, and the diameters of the vessels were expressed by the average figures of these five measurements. Finally, the ratio between the diameter of the venule and that of the arteriole (*v/a* ratio) was calculated. The *v/a* ratios, stressing the importance of venular disease in diabetes mellitus, and giving always figures above 1.0, were preferred to the reciprocal *a/v* values used by Ditzel.

Each person was examined on one occasion only. The photographs obtained from some of the patients showed only one pair of vessels, but in most cases two or even three were to be found. Table 1 shows the results of the determinations of the *v/a* ratios in all the members of the two groups to be studied here. One, two or three *v/a* ratios are noted in columns I-III, according to the number of pairs of vessels that happened to be included on the photographs obtained.

The diameter of the arterioles varied between six and thirty-three micra and those of the venules between twelve and eighty-one micra. These absolute values were calculated by a comparison with a millimeter scale photographed in the same plane as the conjunctiva.

The patients originally consisted of eighty-eight diabetics and thirty control subjects.

In order to exclude vascular changes of old age, the series comprised only persons under fifty years of age; and in order to ensure the necessary cooperation, only persons over ten years were studied. Of the photographs taken, those from twenty patients and six controls were unsuccessful (technical faults or absence of pairs of vessels); accordingly, the final analysis comprises sixty-eight diabetics and twenty-four control subjects. No persons who had previously suffered from severe nondiabetic diseases of the eye (keratoconjunctivitis, iridocyclitis) were included in the study; at the time of the examination, none of the patients or controls showed signs of acute ocular affections.

In order to provide uniform experimental conditions at the time of the examination the following rules were observed: (1) The patient was in the examination room one half hour before the examination was performed; this room was free from draught, the temperature being 20° C. He was not allowed to smoke during this period. (2) The examination was performed at least two hours after the last meal. (3) Alcohol was not allowed during the last twenty-four hours before the examination.

In all patients and including controls, the blood-sugar level was determined by the method of Hagedorn and Norman Jensen before the examination. In the diabetics, the morning and evening urine was tested for sugar, protein and ketone bodies.

The control series consisted of randomly selected healthy persons who had not suffered from serious diseases. As a further precaution, not only blood-sugar determination, but also blood-pressure readings and ophthalmoscopy were performed in the controls. These studies showed normal findings in all.

RESULTS

The results are shown in table 1. A graph of the absolute frequencies of the various *v/a* ratios in the two groups studied (figure 1) gives the immediate impression that high *v/a* ratios are more common in diabetics than in normals. The distribution of the relative frequencies (figure 2) rather intensifies this impression, viz., that the distribution of the values from

TABLE 1

Age, v/a ratios and blood sugar of diabetic patients and of the persons in the control series, divided into age groups. The v/a ratios are the ratios between venular and arteriolar diameter of pairs of vessels in the conjunctiva bulbi. One, two or three pairs of vessels were available for measuring on the photographs obtained from the individual patients and have been listed in columns I-III

D I A B E T I C S							D I A B E T I C S						
Age 11-20 years							Age 41-50 years						
No.	Age	Duration (years)	Pairs of vessels I II III v/a ratios			Blood sugar mg./100 ml.	No.	Age	Duration (years)	Pairs of vessels I II III v/a ratios			Blood sugar mg./100 ml.
002	13	1	2.2	4.6	1.6	165	054	42	17	2.5			318
047	13	8	2.6	1.3		72	076	42	21	1.3	1.2		240
065	15	8	2.2			104	017	43	5	1.5	1.7		87
071	15	0	1.8	1.6		108	018	43	10	2.0	2.0		81
084	15	8	3.5	2.5	2.3	92	021	43	0	1.7			218
052	16	4	2.9	2.6		435	033	43	17	1.2			276
062	16	10	1.6			246	039	43	24	1.3			300
077	16	0	1.7	1.4	1.7	348	053	43	5	1.8	1.6	1.7	250
005	17	1	1.6	3.0		171	087	43	29	2.8			69
064	17	4	3.3			280	020	44	29	1.4	2.8	2.0	114
082	17	4	3.3	2.4	1.8	212	032	44	3	1.5	1.4		156
048	19	2	1.8	4.4		274	069	46	14	2.5	1.3		202
058	20	2	1.9	2.6	1.6		019	47	3	2.9	1.9	3.0	83
041	20	3	1.7	1.5		180	016	48	2	2.1	2.4		110
							026	48	4	1.7			106
							028	49	28	3.0			324
							056	49	12	2.6	2.2		298
							057	49	2	4.1			120
							074	49	2	2.1			75
Age 21-30 years							CONTROLS						
010	21	2	3.3	2.2		467	Age 11-20 years						
059	21	1	1.9	2.4		129							
049	22	10	1.7	2.0	2.1	36	424	12		1.2	1.5		73
055	22	6	1.8			157	423	13		1.6	1.5		77
078	23	1	1.2	2.2		328	413	20		1.4	1.5		114
051	24	22	2.5	1.5	1.5	110	421	20		1.8	1.4		111
070	24	24	2.3	2.2		202	422	20		4.5	1.7		101
009	25	5	1.3	1.9		122							
073	26	20	2.4	1.7		278	Age 21-30 years						
072	27	1	1.1	1.0	4.2	216							
086	30	21	1.3	1.2		200	412	21		1.2	1.1	2.7	104
							400	22		1.9	1.8	1.4	91
							404	23		1.7			79
							415	23		1.3	1.2		79
							416	23		2.3			112
							401	25		2.1	2.3		74
							405	28		1.8	2.0		116
							414	29		1.6	2.1	1.4	103
Age 31-40 years							Age 31-40 years						
022	32	18	1.8	1.6		145							
024	32	5	3.4	2.1			428	31		2.3			145
061	32	3	2.1	3.3	2.0	140	408	32		1.5	1.9	2.6	122
067	32	20	1.1	1.3		67	419	32		2.9	1.2	2.3	90
081	32	26	1.4			262	425	36		2.5	2.4		120
001	35	26	3.1	1.6	2.0	299	411	39		1.7	1.6		94
003	35	22	2.2	1.9	2.0	184							
029	35	25	3.3	1.3		258	Age 41-50 years						
036	35	23	1.4	1.7	1.3	50							
015	36	7	1.6			110	403	42		2.3	1.9	1.8	97
027	36	7	2.5	1.7	1.4	149	417	42		3.3	1.0		80
044	36	16	2.2	1.4		101	429	42		1.4	1.6	2.0	135
045	36	4	2.0	1.3		231	407	44		2.1	1.4	2.2	87
023	37	7	1.7	1.6		264	410	47		2.6			82
035	37	24	2.3	1.2		164	409	48		1.4	1.4		98
038	37	10	3.8			236							
066	37	22	1.5	1.7		279							
060	39	1	1.4	1.7		142							
080	39	2	2.2	2.8		167							
000	40	22	1.9			173							
046	40	12	2.6	4.8	2.2	74							
068	40	16	2.3	1.4		222							
075	40	16	2.9			124							
085	40	0	1.4	1.5		165							

THE V/A RATIO OF THE SMALLER VESSELS OF THE BULBAR CONJUNCTIVA IN DIABETES MELLITUS

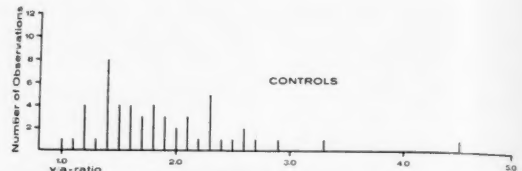
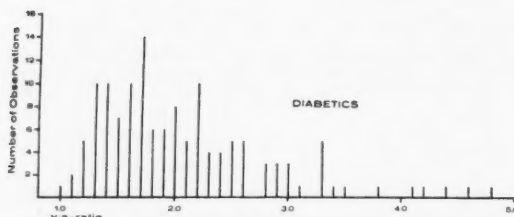


FIG. 1 (a, b). Number of observed v/a ratios in the diabetic and in the nondiabetic control group.

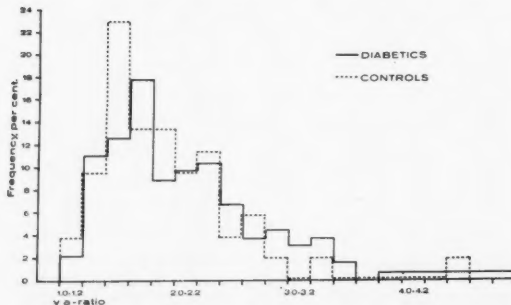


FIG. 2. Frequency of v/a ratios in the two groups, expressed as per cent of the total number of observations in the diabetics (fully drawn line) and in the control group (stippled line). The blocks represent v/a ratio intervals of 0.2.

the diabetics is more positively skew than that of the control group. Analysis of the v/a ratios suggests that the two groups may be characterized by a common mean, but that their variabilities are different, the diabetics being the more variable. However, before all measurements in the two groups are compared, it would seem expedient to make an attempt to eliminate the skewness of the distributions by means of a suitable transformation of the values observed. Taking the logarithms of the observations proved to make the distributions roughly symmetrical so that it may be justified to carry out a statistical analysis based on the assumption of normal or Gaussian distribution. The comparison of the means of the logarithms is as follows:

	Mean	Variance, s^2	Common standard deviation, s
Diabetics	0.2917	0.020897	0.1406
Control group	0.2520	0.016840	
Difference between means:		0.0397	
Standard deviation of the difference:			

$$0.1406 \sqrt{1/135 + 1/52} = 0.0229$$

$$t : 0.0397 / 0.0229 = 1.734$$

On the hypothesis that the true mean in the diabetic group must be either equal to or larger than that of the control group the difference between the means is significant (the value of t calculated lies between the

95 and the 97.5 per cent fractiles of the distribution of t or, equivalently, between the 5 and 2.5 percentage points). In the calculation of t a common variance, s^2 , has been assumed, which may be judged to be permissible, since the two s^2 -values are not significantly different.

An analysis of variance was performed on the two groups in order to separate inter- and intra-individual variation, showing that whereas the interindividual variation was larger than the intra-individual variation in the diabetic group, the opposite relation characterized the control group, i.e., that different measurements in the same person of this group were more variable than the means of different persons. Although not significant this relation is so unusual that it is judged to be worth mentioning. If, instead of the above common value of s for the total distribution, a value of s calculated from the interindividual variances is used, the t value becomes lower (1.668), since s is then increased from 0.1406 to 0.1462, but still t lies between the 95 and the 97.5 per cent fractiles.

By inspection of table 1, one suspects a higher incidence of high v/a ratios in the young, but a statistical analysis of the relation between age and v/a ratio showed no significant correlation between these two characteristics, nor was any correlation between duration of diabetes and v/a ratio proved beyond doubt. At any rate nothing suggested a linear relationship between the v/a ratio and the duration of diabetes. We rather gained the impression that an initial increase was followed first by a decrease and then another increase in the v/a ratio during the first thirty years of the diabetes. This, however, cannot be taken as positively demonstrated. However, the aforementioned fact that the interindividual variation in the diabetics is larger than in normal subjects suggests that some relationship may exist between the v/a ratio and the development of the disease over the course of years. However, as indicated by the t -test above, the difference between the inter-individual variances is not statistically significant in the present series.

The subdivision of our data by age and duration of

diabetes gives rather small groups. Investigations including larger number of patients are necessary to elucidate further the relation between the v/a ratio and the age and duration of diabetes.

Five of the patients had slight ketonuria at the time of examination (Nos. 010, 022, 023, 066, 077). It appears from table 1 that the v/a ratios observed in these cases were not particularly high.

DISCUSSION

The results of the present quantitative study confirm the observations reported by Ditzel et al., viz., that the v/a ratio of the conjunctival vessels is often higher in diabetics than in nondiabetics.

It is interesting that this should be demonstrable even in spite of the rather large intra-individual variation. The v/a ratio of one pair of vessels may be rather different from that of another pair of vessels in the same conjunctival bed. All the same the statistical analysis of the results obtained shows that high ratios occur more often in a group of diabetic than in a group of nondiabetic eyes.

The difference between the v/a ratio in diabetics and in nondiabetics is statistically significant, but it is, of course, not possible to diagnose or exclude diabetes mellitus by this ratio.

It should also be emphasized that the differences found in our series seem to be less pronounced than those reported by Ditzel. Ditzel and Sagild⁹ found v/a ratios over 3:1 in 48 per cent of young, in 28 per cent of middle-aged and in 6 per cent of older diabetics. In control subjects ratios over 3:1 were never observed. In children, Ditzel and Duckers¹¹ found v/a ratios of 2:1-3:1 in 53 per cent, of 4:1 in 29 per cent, of 5:1 in 14 per cent, and ratios equal to or greater than 6:1 in 4 per cent. Only 1 per cent of the control subjects had a v/a ratio above 3:1.

Like Ditzel and his associates, we found that high v/a ratios occur both in short-term and in long-term diabetes. The question is therefore: What connection is there between the peculiarities of the conjunctival vessels in diabetics and the typical incapacitating angiopathy of long-term diabetes?

It is reasonable to assume that long-term diabetic vascular disease, characterized by the well-known histological abnormalities and clinical manifestations, represents the end result of a development which has been going on ever since the beginning of the diabetes. The relative venular dilatation observed in our patients with diabetes of a few years' duration demonstrates a vascular anomaly in this clinically latent period before the ap-

pearance of gross vascular damage.

Information about the state of the blood vessels in the first years of diabetes mellitus is still scarce. Bárány¹⁸ studied diabetic patients without signs of vascular occlusion and often found a reduction of heat dissipation during body warming as well as of the intracutaneous Na²⁴ clearance. Sigroth¹⁹ studied skin temperature in diabetic patients during indirect heating and in post-ischemic hyperemia. Reduced vascular responses were found, and improvements of responses occurred in several cases when a better control was obtained. Butterfield¹⁷ found that the foot blood flow, measured plethysmographically, was usually low in diabetic patients, also in patients with short duration of the disease. Finally, Steiness¹⁸ has demonstrated that diabetic patients maintain their vibratory perception longer than normal after arrest of the blood flow to the limbs. This abnormality was observed both in patients with recent diabetes and in diabetes of long duration; it seems to be reversible in most patients. It should be mentioned also that Ditzel and Camerini-Davalos¹⁸ have reported rapid amelioration of some of the vascular changes in the conjunctiva when a poor diabetic state was brought under control.

Further studies of the blood vessels in the early years of diabetes are needed. More information about the apparently reversible functional changes of the vascular bed in recent diabetes mellitus might help us to a better understanding of long-term diabetic vascular disease, and, eventually to appropriate measures for their prevention.

SUMMARY

The bulbar conjunctiva was photographed by a standardized experimental setup in sixty-eight diabetics and twenty-four nondiabetics. The arterioles and accompanying venules were measured on the negatives by means of a measuring microscope, following which the v/a ratio was calculated.

Statistical analysis of the numerical material obtained showed that high v/a ratios were more frequent in diabetics than in nondiabetics.

ACKNOWLEDGMENT

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SUMMARIO IN INTERLINGUA

Le Proportion Venulo-Arteriolar del Conjunctiva Bulbar in Diabete Mellite

Le conjunctiva bulbar esseva photographate per un

standardisate methodo experimental in sexanta-octo diabeticos e vinti-quattro non-diabeticos. Le arteriolas e le accompagnante venulas esseva mesurate in le photographias per medio de un microscopio mesurante, e postea le proportion venulo-arteriolar esseva calculate.

Le analyse statistic del magnitudes obtenite monstrava que alte proportiones venulo-arteriolar esseva plus frequente in diabeticos que in non-diabeticos.

ADDENDUM

Since the submission of this manuscript J. Ditzel, D. W. Beaven and A. E. Renold have published "Early Vascular Changes in Diabetes Mellitus" (*Metabolism* 9:400, 1960), containing among other things a short report of a quantitative examination of the conjunctival vessels in diabetics and nondiabetics. The difference between the distribution of v/a ratios in diabetics and in nondiabetics, calculated from their original data, is essentially similar to the one reported here (J. Ditzel, personal communication).

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The Metabolism of Adrenocortical Hormones in Pregnancy

It is widely recognized that many important alterations in metabolism occur in pregnancy which are determined by ovarian, pituitary, placental and adrenal cortical hormones. There is now unfolding a body of information indicating that the metabolism of hormones themselves often is decidedly different in the pregnant state.

Measurement of the urinary excretion of corticosteroids has usually provided important information concerning corticosteroid metabolism. An increase in the excretion of glyconic corticoids in the latter half of pregnancy was reported by E. Venning (*Endocrinology* 39:203, 1946). Subsequent measurements of free cortisol excretion in pregnancy is consistent with this finding. A number of other workers who measured the

urinary excretion of corticosteroids with chemical methods, which included both the free and the conjugated metabolic products of cortisol, found that there was no significant increase in the excretion of total corticosteroids.

When methods became available for the measurement of unconjugated 17-hydroxycorticosteroids, mainly cortisol, in the blood, a significant rise to about twice the normal level was observed during the third trimester in pregnant women. An exaggerated rise in the plasma cortisol takes place after the administration of ACTH.

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Effects of Insulin on Blood Glucose Entry and Removal Rates in Man

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By measuring the rate of decline of the specific radioactivity of blood glucose following injection of a single dose of glucose uniformly labeled with carbon-14, one can calculate its rates of entry to and removal from the blood.¹ In applying this procedure in experiments with intact dogs and human subjects results were obtained that indicated that the immediate hypoglycemic action of insulin is due not only to an accelerated removal, but also to a lowered inflow of blood glucose.^{1,2} However, the relative quantitative importance of each of these effects is still uncertain. Inasmuch as the liver is in all likelihood the principal source of the blood glucose, and since this organ has a considerable capacity for binding insulin,³⁻⁶ it may be anticipated that hepatic effects of insulin might predominate during its slow introduction into the blood, such as would occur when it is physiologically secreted. It has been reported^{7,8} that intraportal injection of insulin is not as effective in lowering blood sugar as peripheral venous injection. Moreover, the oral hypoglycemic agents, whose effects are generally attributed to their ability to stimulate pancreatic insulin secretion, apparently suppress hepatic glucose output but have little effect on peripheral glucose utilization.⁹⁻¹² To gain further information on the possible quantitative importance of an hepatic effect of insulin, a series of experiments was conducted in which a slow, steady introduction of insulin into the bloodstream was established by subcutaneous injection of the hormone.

The results of the earlier studies² were extended and confirmed in indicating that a slow, regular entry of insulin into the blood prolongs the hepatic effect and decreases the peripheral action as compared with a single intravenous injection.

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EXPERIMENTAL PROCEDURE

The subjects in the present study were middle-aged and elderly patients of a home for the aged and the clinic of a large community hospital (table 1).^{*} Criteria for their selection, and general procedures for injection, blood withdrawal, analyses, and radioactivity assay are given in previous publications.^{1,2} While the subject was at rest on a cot or comfortable arm chair, the glucose in a "trace" dose (approximately 20 mg.) of approximately 100 microcuries, dissolved in 10 ml. of sterile saline solution, was injected in an arm vein. Samples of blood were then drawn at fifteen- to twenty-minute intervals for periods of fifty to 100 minutes in order to allow for equilibration and to obtain a pre-insulin glucose turnover rate. Glucagon-free insulin (lot number T-3194-C†), freshly prepared in a concentration of 20 units per milliliter in sterile isotonic saline solution was then injected subcutaneously in an arm, and blood sample collections were continued for periods of two hours or longer, at first at five- to ten-minute intervals, then at fifteen- to thirty-minute intervals. During the course of the experiment, the patients were allowed coffee, sweetened with saccharin, or water, ad lib.

The present report covers data on the effects of subcutaneously injected insulin in sixteen patients. These are reported in several charts which illustrate the types of response obtained in individual instances; and in a summary table which includes all experiments of this type conducted thus far. To appreciate the action of insulin in these experiments it is necessary to consider the normal curve of radioactivity decline following intravenous injection of glucose-C-14. In figure 1 there are given the results of an experiment in which glucose

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†Kindly supplied by W. R. Kirtley, M.D., of the Lilly Research Laboratories, Indianapolis, Indiana.

TABLE 1

Subjects of investigation of insulin action

Patient	Wt. kg.	Age	Sex	Diagnosis
T.O'B.	40	64	M	1. Carcinoma of esophagus
S.I.	72	84	M	1. Arteriosclerotic heart disease 2. Carcinoma of prostate 3. Peripheral vascular disease
A.S.	82	76	M	1. Arteriosclerotic heart disease 2. Chronic congestive heart failure 3. Laennec's cirrhosis with anemia
I.P.	78	68	M	1. Diabetes mellitus of four years' duration; no insulin
I.S.	50	77	M	1. Arteriosclerotic parkinsonism 2. Essential hypertension
D.A.	101	43	F	1. Diabetes mellitus of three years' duration; Orinase, 0.5 gm. four times daily 2. Right lower lobar pneumonia
E.K.	60	39	F	1. Laennec's cirrhosis—early
B.H.	54	68	F	1. Arteriosclerotic vascular disease
I.M.	53	78	M	1. Inactive pulmonary tuberculosis 2. Irritable colon syndrome
N.Z.	69	80	M	1. Arteriosclerotic heart disease 2. Pulmonary emphysema
H.B.	79	86	M	1. Arteriosclerotic heart disease 2. Chronic peptic ulcer
S.G.	78	78	M	1. Arteriosclerotic heart disease 2. Chronic bronchial asthma
I.L.	68	83	M	1. Arteriosclerotic heart disease
I.K.	45	82	M	1. Chronic peptic ulcer 2. Hypertensive cardiovascular disease
S.S.	59	64	M	1. Essential hypertension 2. Arteriosclerotic heart disease 3. Arteriolar nephrosclerosis

turnover was measured in a seventy-five-year-old man with no obvious endocrine disorder or disturbance of carbohydrate metabolism. During the course of the six-hour period of blood collection the blood sugar remained highly constant at 110 mg. per 100 ml. In this, as in most of our experiments on glucose turnover, the decline in specific activity of the blood glucose is best described by a biphasic curve. Up to 150 to 180 minutes (and neglecting values obtained prior to thirty minutes after the injection of the labeled glucose) there is a strictly exponential drop in specific activity. In the light of the constancy of the fasting blood sugar levels of these subjects, it is assumed that the decline signifies a constant rate of replacement of the blood glucose during this interval, which can readily be calculated from the first order reaction rate expression.² In this experiment the initial turnover rate, R , was 0.67 mg. per 100 ml. per minute. After 150 to 180 minutes there is a tendency for the slope of the specific activity

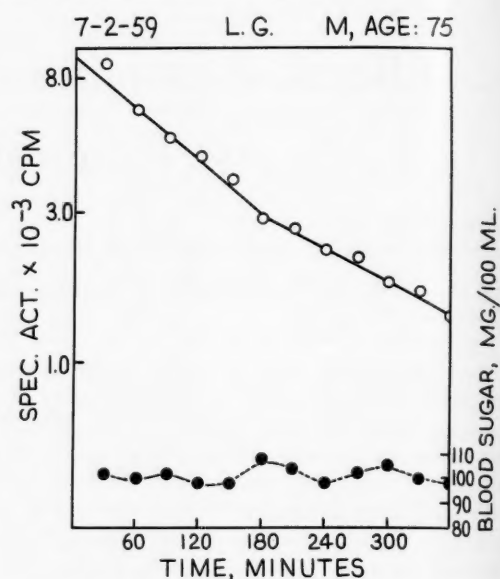


FIG. 1. Blood glucose turnover in a nondiabetic man (L.G.). Blood sugar level, broken line, right ordinate; specific activity, solid line, left ordinate plotted on a logarithmic scale. Radioactive glucose was administered at time zero.

curve to decline slightly. From the line best fitting the data between 160 and 360 minutes the calculated turnover rate during this latter interval was 0.49 mg. per 100 ml. per minute. This pattern is typical of most of our experiments, though the decline in slope was not always observed.

By extrapolating the initial specific activity curve back to zero time, one can obtain the "zero time" dilution of the injected glucose, and thus calculate the total body glucose pool.¹ In the present experiment the value was 20.4 gm. corresponding to 345 mg. per kilogram. From the observed glucose concentration of 110 mg. per 100 ml., the glucose space was 18.6 L. or $18.6 \times 100/59.1$ kg. = 31 per cent of the body weight.

In a total of twelve experiments of this type carried out thus far in individuals with no obvious disorder of glucose metabolism the average glucose turnover rate was 0.58 mg. per 100 ml. per minute, the pool size was 320 mg. per kilogram, and the space was 32 per cent of the body weight. These values are only regarded as approximations of the order of magnitude, however, since the numerous assumptions required in making the calculations are probably not wholly valid (see for example,¹³⁻¹⁵). A more detailed study of glucose turnover

in the human will be published separately.

Response of blood glucose to subcutaneous insulin injection. Results of individual experiments are portrayed graphically in figures 2, 3 and 4 which illustrate the various responses obtained to subcutaneous insulin injection. The experiment shown in figure 2 was conducted on a sixty-four-year-old man suffering from essential hypertension, arteriosclerotic heart disease and arteriolar nephrosclerosis. Several notable differences from previous experiments with intravenously injected insulin² were observed. With subcutaneous insulin in the dosage of 0.2 units per kilogram there is usually a delay of from ten to forty minutes before the blood sugar begins to drop, and the drop, though not as rapid nor as deep, is more prolonged. There is, similarly, a delay in the break in the specific activity, but just as with intravenous insulin injection, there is a close coincidence in time between the "plateauing" of specific activity and the onset of hypoglycemia. In this experiment the plateau covered a period of forty-five minutes, in marked contrast with the action of intravenous insulin, in which it usually lasted only ten to twenty minutes. If the experiments were sufficiently prolonged, as in figure 2, a period of recovery or stabilization was observed following the hypoglycemic stage. During this

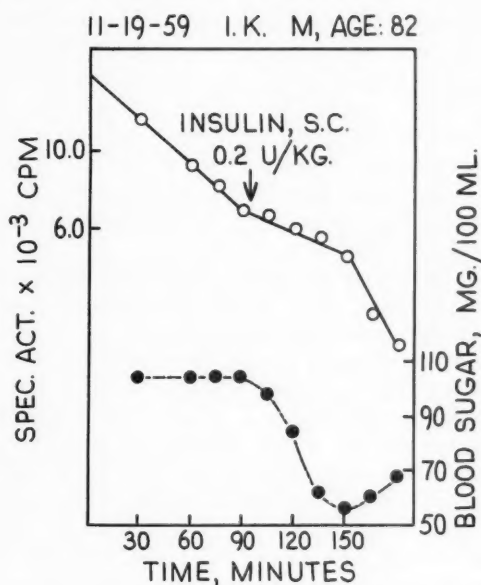


FIG. 3. Same experiment as figure 2 with a nondiabetic man (I.K.) given 0.2 U insulin per kilogram.

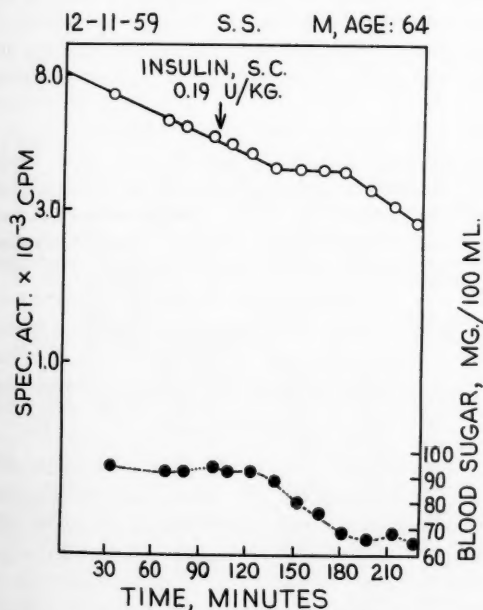


FIG. 2. Time course of blood glucose concentration and specific activity plotted as in figure 1. Arrow indicates time of subcutaneous administration of insulin in a dose of 0.19 U per kilogram. Nondiabetic man (S.S.).

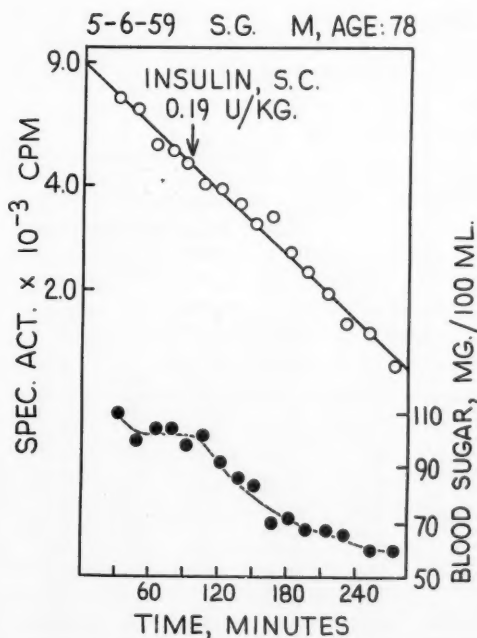


FIG. 4. Same experiment as figure 2 with a nondiabetic man (S.G.) given 0.19 U insulin per kilogram.

time the specific activity resumed a more rapid downward trend, indicative of a renewed release of hepatic glucose, while the blood sugar was constant at about 65 mg. per 100 ml. The turnover rate was 0.55 mg. per 100 ml. per minute during this time, a value near the pre-insulin turnover rate. Unfortunately, in this as in most of these experiments, the time could not be extended long enough to include the subsequent period of recovery of blood sugar level.

In figure 3 are data illustrative of eight experiments in which there was a partial suppression of glucose entry. For the first ninety-four minutes prior to insulin injection there was a constant blood sugar level of 104 mg. per 100 ml., and an exponential drop in specific activity corresponding to a turnover rate of 1.05 mg. per 100 ml. per minute. During the next fifty-five minutes after subcutaneous insulin injection the specific activity curve had a decreased slope and the blood sugar level dropped from 105 to 57 mg. per 100 ml. Entry and removal rates during this period, calculated according to the previously described procedures, were, respectively, 0.32 mg. per 100 ml. per minute, and 1.26 mg. per 100 ml. per minute.

During the ensuing recovery period of thirty minutes the respective rates were 1.26 and 0.83 mg. per 100 ml. per minute.

Figure 4 illustrates two experiments in which no change in slope was discerned following subcutaneous insulin injection in which the calculated drop in blood

entry was not significant. Also, little effect was seen on the blood sugar; there was a gradual drop from 100 to 60 mg. per 100 ml. over a two-hour period.

Table 1 presents data obtained in all sixteen experiments in which the action of subcutaneous insulin was examined. They were carried out at intervals over a two-year period, and are given in chronological order. In all of these, blood sugar levels were reasonably constant throughout the pre-insulin period ranging from forty-six to 130 minutes. The turnover rate of the blood glucose during this period ranged from 0.35 to 1.4 mg. per 100 ml. per minute, with an average rate of 0.72 mg. per 100 ml. per minute. After a variable lag period following subcutaneous injection of insulin, presumably the time required for sufficient insulin to enter the blood and to reach the affected tissue, the blood sugar began to drop, and the specific activity declined less rapidly. The drop in blood sugar in the different experiments ranged from 10 to 72 mg. per 100 ml. and the time during which this drop occurred ranged from thirty to 180 minutes.

In experiments eight and fourteen, in which insulin effects were minimal, its effects on the blood sugar were also small.

In eight experiments there was an essentially complete "plateauing" of slope lasting from thirty to 120 minutes in the different experiments. No correlation was observed between the "pre-insulin" turnover rate and the degree of suppression of glucose output. In

TABLE 2
Effects of subcutaneous insulin on blood sugar entry and removal rates

Date	Patient	Pre-insulin period				Hypoglycemic period				Stabilization and recovery			
		Time	Blood sugar	Replacement rate*	Insulin	Time	Blood sugar change	Entry rate*	Removal rate*	Time	Blood sugar change	Entry rate*	Removal rate*
		minutes	mg. per 100 ml.		U/kg.	minutes	mg. per 100 ml.			minutes	mg. per 100 ml.		
9-30-57	T.O.B.	0-48	115	1.1	0.25	48-144	—35	0.62	0.98				
11-4-57	S.I.	0-47	106	1.1	0.14	58-110	—52	0.0	1.0	110-140	+14	0.90	0.43
10-14-57	T.O.B.	0-46	125	0.4	0.38	47-77	—25	0.0	0.8	77-107	—30	0.55	1.60
10-22-57	A.S.	0-48	117	0.9	0.15	48-98	—29	0.3	0.9				
10-25-57	I.P.†	0-48	285	1.4	0.13	62-130	—60	0.0	0.9				
12-16-57	I.S.	0-48	112	0.9	0.17	58-90	—37	0.0	0.9	90-126	0	0.75	0.75
2-18-59	D.A.†	0-80	173	0.7	0.10	105-225	—72	0.0	0.6				
3-12-59	E.K.	0-100	135	0.95	0.10	100-180	—40	0.65	1.2				
3-19-59	B.H.	0-80	100	0.50	0.11	80-145	—50	0.20	0.95	145-185	0	0.90	0.90
4-2-59	I.M.	0-80	70	0.60	0.15	80-120	—20	0.15	0.65				
7-22-59	N.Z.	0-130	100	0.6	0.15	140-200	—37	0.26	0.88	200-240	0	0.70	0.70
7-22-59	H.B.	0-125	105	0.6	0.14	125-150	—10	0.0	0.4				
8-6-59	S.G.	0-90	104	0.7	0.19	90-270	—40	0.55	0.74				
8-20-59	I.L.	0-90	96	0.7	0.19	106-136	—22	0.0	0.7				
11-19-59	I.K.	0-94	104	1.1	0.20	94-150	—50	0.4	1.3	150-180	+13	1.3	0.8
12-11-59	S.S.	0-100	92	0.5	0.19	140-180	—20	0.0	0.5	180-226	0	0.6	0.6

*Values in these columns are given in mg. per 100 ml. whole blood per minute.

†Mild diabetes.

seven other experiments the specific activity curves decreased in slope, but did not "plateau," pointing to a partial suppression of release. This partial suppression of glucose inflow varied widely; in experiment one, the rate of entry, R , fell from 1.1 to 0.65 mg. per 100 ml. per minute. In two other experiments, the fall was less than 30 per cent, e.g., in experiment eight from 0.95 to 0.65, and in experiment fourteen, from 0.74 to 0.55 mg. per 100 ml. per minute. In the other four experiments in which a partial suppression of glucose inflow occurred, the suppression ranged from 57 to 75 per cent; again there was no correlation with initial turnover rate.

In contrast with the short-lasting effects of intravenous insulin² the action of subcutaneous insulin was prolonged. In the eight experiments in which "plateauing" occurred, the effect lasted from thirty to 120 minutes; in the other experiments in which there was partial inhibition of glucose inflow, the effect lasted forty to 180 minutes.

A significant difference from the results obtained with intravenously injected insulin was noted in its effect on the outflow rates of blood glucose. Of the sixteen experiments shown, it was not affected materially in eight, it was increased moderately in five, and it was decreased moderately in three experiments. In only two of the sixteen experiments was the outflow rate as much as doubled, whereas the intravenous injection of insulin invariably led to increases in outflow rates of threefold or higher.^{1,2}

In seven experiments the total elapsed times were sufficiently long to include a period of recovery or stabilization of the blood sugar level; during this interval, rates of inflow and outflow generally returned to levels characteristic of the initial, pre-insulin period.

Two of the subjects were mildly diabetic. One of these exhibited the highest observed turnover rate, the other an average rate. Both exhibited insulin sensitivity, marked by large depressions in blood sugar and "plateauing" of specific activity after insulin injection.

DISCUSSION

In our previous publications^{1,2} evidence of other investigators for and against a suppressive action of insulin on hepatic glucose release was summarized. This field continues to be controversial; with the publication of new material there is still a wide divergence of opinion concerning the existence of the plateauing effect, and of its interpretation.¹⁸⁻²¹ Shoemaker et al.^{10,19} carried out experiments using combined technics of C-14-labeling and measurement of plasma glucose gradients in

dogs. Though "plateaus" in plasma glucose C-14 activity were observed following insulin injection, the gradient of plasma glucose level across the liver, as measured by simultaneous sampling of portal and hepatic venous blood, was not decreased. Although the total splanchnic glucose output was decreased by insulin, this was reported to be due to increased uptake of glucose by non-hepatic splanchnic tissue. On the other hand, Madison and Unger in similar experiments with dogs,²² reported that the endoportally administration of insulin decreased the portal-hepatic gradient of plasma glucose, and had a lesser effect on the peripheral arterial-venous gradient than systemically administered insulin. They concluded that insulin decreased hepatic output and in agreement with the result here presented, suggested that when administered endoportally, the hepatic effect predominated over the peripheral effect.

More evidence of an hepatic action of insulin, as well as a further clarification of the action of the hypoglycemic drugs has been brought forward by Frawley et al.¹¹ These investigators had shown previously that whereas insulin markedly increased the disappearance of infused D-xylose from the blood of human subjects, no such effect occurred with tolbutamide. However, when insulin was injected intraportally it too had only a minimal effect on xylose disappearance. In hepatectomized dogs, both insulin and tolbutamide increased uptake of glucose in peripheral tissues. These investigators interpret these results as indicating a predominant hepatic effect of endogenous insulin, which is due both to its slow release, and its traversal of the liver before entering the general circulation. This view coincides precisely with our interpretation of our previous results with tolbutamide,³ and receives additional support from our present experiments.

Searle et al., in experiments in humans similar to those previously reported by us,¹⁸ were in general agreement with the view that insulin (and tolbutamide) suppress hepatic glucose release. On the other hand, Tarding and Schambye¹⁹ in experiments similar to ours observed plateaus with tolbutamide but not with insulin, and since these investigators found the same hepatic effect of tolbutamide in depancreatized dogs, they concluded that this substance depressed hepatic glucose release independently of insulin, which they concluded had no such effect itself.

The de Bodo group has conducted an extensive investigation of blood glucose inflow and outflow rates in dogs, using a technic similar in principle to ours, but differing in that the glucose-C-14 is infused continuously at a rate sufficient to balance the inflow of unlabeled

glucose, so as to maintain a constant specific activity under steady-state conditions.²⁰ Although a transient suppressive action of insulin on glucose inflow was observed when given in a single intravenous dose,²⁰ this was not regarded as an important factor in the blood sugar regulation. More recently, however, this group has conducted continuous intravenous infusions of insulin in dogs and observed that the normal hepatic response to hypoglycemia, viz., a release of glucose into the bloodstream, does not occur until the insulin infusion is terminated.²³ More recently another suppressive action of insulin on hepatic glucose release was reported.²⁴ To what extent these hepatic actions of insulin are related to the effect observed by us remains to be determined.

The present experiments involving subcutaneous insulin injection reinforce our previous data which indicated a suppressive action of insulin on hepatic glucose release.^{1,2} They differ from these in two particulars. First, the period of "plateauing" of blood glucose specific activity, which we believe results from suppressed hepatic glucose release, is considerably prolonged; second, the marked increase of peripheral glucose utilization following the single intravenous injection of insulin was not observed. Without denying an important role of insulin in the peripheral utilization of glucose, particularly during hyperglycemia, we believe the present results, in conjunction with previous investigations, indicate that normoglycemia in the basal state is maintained by regulation of hepatic glucose release no less than by peripheral glucose uptake, and that insulin plays a role in both processes.

SUMMARY

Two mild diabetic and fourteen nondiabetic humans were given a "trace" dose of uniformly C-14-labeled glucose and blood samples were removed at frequent intervals before and after the subcutaneous administration of insulin. Before insulin injection the logarithmic drop of specific activity coincident with a constant blood glucose concentration indicated a constant turnover rate of the blood glucose which averaged 0.72 mg. per 100 ml. per minute. Following the subcutaneous injection of insulin and coincident with the fall of the blood sugar level the specific activity either "plateaued" or declined less rapidly. In eight experiments there was an essentially complete "plateauing" of slope indicative of complete suppression of hepatic glucose output. In the remaining experiments the rate of glucose entry varied widely, ranging from 30 to 75 per cent of the pre-insulin rate. The effect of subcutaneous insulin ad-

ministration on the outflow rates was significantly different from that of the intravenously injected hormone. Of the sixteen experiments presented it was not affected significantly in eight, it was increased moderately in five, and it was decreased moderately in three. The present experiments involving subcutaneous insulin injection reinforce our previous data and emphasize a role of insulin in regulating hepatic glucose output during normoglycemia and hypoglycemia.

SUMMARIO IN INTERLINGUA

Le Effectos de Insulina Super le Movimento de Glucosa in e ex le Sanguine de Humanos

Duo levemente diabetic e dece-quattro non-diabetic subjectos human recipeva doses "traciatori" de glucosa a marcation uniforme con C-14, e specimens de sanguine esseva obtenite a frequente intervallos ante e post le administration subcutanee de insulina. Ante le injection de insulina, le cadita logarithmic de activitate specific coincidente con un concentration constante de glucosa sanguinee indicava un constante rapiditate del movimento de glucosa in e ex le sanguine, amontante a un valor medie de 0,72 mg per 100 ml per minuta. Post le injection subcutanee de insulina e coincidente con le cadita del nivello de sucro in le sanguine, le activitate specific manteneva un plateau o declinava minus rapidamente. In octo experimentos il occurreva un essentialmente complete "plateauification", indicante le suppression total del rendimento hepatic de glucosa. In le altere experimentos, le mesuras del entrata de glucosa variava extensemente, con valores de inter 30 e 75 procento del mesura ante le injection de insulina. Le effecto de insulina subcutanee super le intensitate del effluxo differeva significativamente ab le effecto de insulina intravenose. In le caso de insulina subcutanee, octo del reportate dece-sex experimentos non demonstrava un effecto significative super le supra-mentionate parametro; in cinque iste valor esseva moderatemente augmentate; in tres illo esseva moderatemente reduce. Le presente experimentos con injectiones de insulina per via subcutanee reinforta nostre previe datos e sublinea le signification del rolo de insulina in le regulation del rendimento hepatic de glucosa durante normoglycemia e hypoglycemia.

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Metabolism and Relative Hypoglycemic Potencies of Four Sulfonylureas in Man

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This paper presents the results of studies in which the relative potencies of tolbutamide, carbutamide, chlorpropamide and metahexamide were determined immediately after intravenous administration. The relationship between these acute potencies and the clinical potencies of these drugs is discussed in the light of previous findings concerning the differences in the metabolism of these drugs. In addition, the results of further studies concerning the rates of absorption of these drugs are presented.

In previous studies¹⁻⁴ concerning the comparative pharmacology of sulfonylureas in man, we attempted to estimate the clinical potencies indirectly by determining the potencies during the first few hours after oral administration of the drugs and by investigating the metabolic half lives of the drugs. However, in these and other studies⁴ we found that under some conditions there were substantial differences in the rates of absorption of certain sulfonylureas. These differences may result in erroneous interpretations of the results of some types of experiments. For example, it has become apparent that our previous finding that chlorpropamide was approximately twice as potent per milligram as tolbutamide during the first few hours after ingestion² was not due to the inherently greater potency of chlorpropamide, but rather to its greater rate of absorption.⁴ There is still another reason why it may be difficult to determine relative potencies of sulfonylureas by means of experiments in which only the acute hypoglycemic responses of normal subjects or normal animals to orally administered drugs are compared. The blood glucose levels observed under these conditions are not necessarily related exclusively to the potencies of the doses administered, since certain subjects may exhibit rises in blood glucose secondary to counter-regulatory responses to the induced hypoglycemia, thus obscuring the drug effect.

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For these reasons we have investigated the potencies of a series of sulfonylureas immediately after intravenous injection. Our preliminary studies and those of Unger and Madison⁵ showed that after the intravenous administration of tolbutamide hypoglycemic responses occurred promptly and that during the first thirty minutes after injection progressive hypoglycemia occurred. In normal subjects, thirty to sixty minutes after injection of 1 gm. of tolbutamide, a rise in blood glucose occurred which was presumably due to a counterregulatory response to hypoglycemia. It seemed, then, that measuring the fall of the blood glucose during a thirty-minute period after the intravenous injection of a sulfonylurea derivative might be a satisfactory way of determining its relative potency.

METHODS

The blood glucose levels were determined on venous blood by the method of Nelson.⁶ The levels of metahexamide and carbutamide in blood were determined by the method of Bratton and Marshall⁷ with the minor modification described previously.⁴ The levels of tolbutamide and chlorpropamide were determined by the method of Toolan and Wagner.⁸ A minor modification⁴ of this method was employed in determining tolbutamide concentrations because of its slightly different absorption curve.

EXPERIMENTAL RESULTS

Potencies of sulfonylureas after intravenous administration. In table 1 are shown the results of experiments in which the relationship of response to dose was tested by observing the fall in the fasting blood glucose thirty minutes after the intravenous injection of various doses of tolbutamide. Each of five normal subjects received in random order five different doses of tolbutamide at intervals of forty-eight to seventy-two hours. It may be noted that the small responses to 125 mg. were not much greater than to 63 mg. However, as the dose was progressively increased from 125 mg. there was a consistent relationship between dosage and response. The data in table 1 show that, except on one occasion in a single subject, raising the dose above 125 mg. always increased the response.

TABLE 1

Relative potencies of five hypoglycemic agents in normal subjects

Decrease in blood glucose in mg. per 100 ml. 30 min. after I.V. injection						
Subject	1	2	3	4	5	Mean (mg. per 100 ml.)
Tolbutamide						
63 mg.	8	8	14	11	6	9
125 mg.	10	6	17	7	10	10
250 mg.	9	18	27	28	15	19
500 mg.	14	20	31	33	26	25
1,000 mg.	36	32	34	41	36	36
Chlorpropamide						
250 mg.	12	17	18	18	21	17
Carbutamide						
500 mg.	16	13	28	18	13	18
Meta-hexamide						
63 mg.	11	15	17	21	18	16
250 mg.	25	23	37	45	34	33
Insulin (glucagon-free)						
2 Units	22	20	27	29	6	21

From these data it was possible to construct a standard dose-response curve with which the potencies of other hypoglycemic agents could be compared (figure 1). Based on preliminary observations, doses of other drugs to be tested were selected so that the responses might fall on that part of the tolbutamide curve in which there was a good correlation between dosage and response. In figure 1 are summarized the results of such experiments in which the mean responses of the five subjects who had received five different doses of tolbutamide were compared with their responses to other drugs. The individual data are shown in table 1. Chlorpropamide, 250 mg., produced responses which were not significantly different from those produced by 250 mg. tolbutamide. On the other hand, carbutamide, 500 mg., produced responses which were far less than those produced by 500 mg. of tolbutamide. The blood sugar responses to 500 mg. of carbutamide were similar to those exhibited by these subjects to 250 mg. of tolbutamide. Two different doses of meta-hexamide were administered to each of the five subjects. A dose of 63 mg. produced responses only slightly less than those produced by doses of tolbutamide four times larger. Similarly, doses of meta-hexamide of 250 mg. produced responses only slightly less than those exhibited by these same subjects after 1,000 mg. of tolbutamide. Two units of glucagon-free insulin produced responses approximately equal to those pro-

POTENCIES OF HYPOGLYCEMIC AGENTS COMPARED TO TOLBUTAMIDE

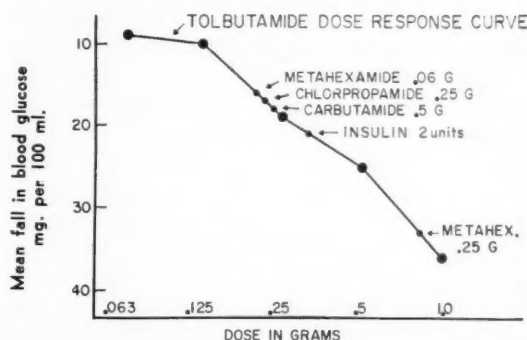


FIG. 1. The line represents a dose-response curve of tolbutamide constructed from the data in table 1. The mean responses of five subjects to meta-hexamide, chlorpropamide, carbutamide, and glucagon-free insulin are plotted in relationship to the curve formed by the mean responses of the same subjects to various doses of tolbutamide. Each response is the difference between the control blood glucose and that thirty minutes after intravenous administration.

duced by 250 mg. of tolbutamide.

It should be emphasized that the response of each experimental subject to each dose of each drug was determined. This approach made it possible to identify significant differences among the responses after the fewest possible tests using a formula for paired data.¹⁰ For example, that 250 mg. of meta-hexamide was more potent than 500 mg. of tolbutamide was established statistically because each of the five subjects exhibited a greater response to the smaller dose of meta-hexamide ($p < .01$). Similarly, it was shown that 250 mg. of chlorpropamide did not produce responses as great as those produced by 500 mg. of tolbutamide ($p < .05$). A statistical comparison of the responses to 500 mg. of carbutamide with the greater responses to 500 mg. of tolbutamide revealed a difference of borderline significance ($p = .09$).

Absorption rates of sulfonylureas. The data summarized in figure 2 show that the rate of absorption of tolbutamide, based upon serum levels one and two hours after administration of a test dose, was much slower than the rate of absorption of meta-hexamide and chlorpropamide when pulverized tablets were administered in warm coffee. Absorption of carbutamide was a little slower than that of meta-hexamide and chlorpropamide. The serum levels of tolbutamide at both one and two hours are significantly lower than the levels of each of the other drugs ($p < .05$ in all instances). Our previous data⁹ suggest that eventually the absorption of tolbutamide is virtually complete. We

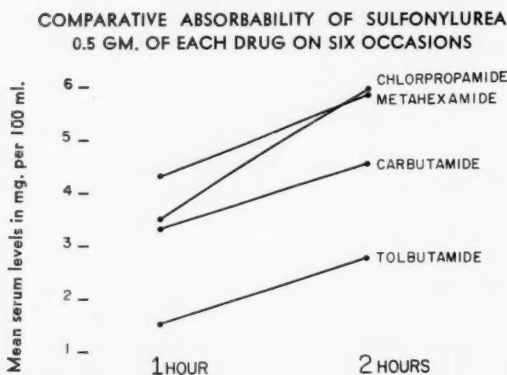


FIG. 2. Each of the drugs was administered as a pulverized tablet to each of six subjects. The mean serum levels of drug are represented.

found that after intact tablets were administered (three subjects), and after pulverized tablets were administered (three subjects), serum tolbutamide levels were initially (one and two hours) much lower, but later (four hours) only slightly lower than serum levels of the other three drugs.

It is possible that the rate of absorption of a sulfonylurea is related to its solubility. It is of interest in this connection that at a *pH* similar to that in the small intestine (7.0) the solubility of chlorpropamide is greater than carbutamide which, in turn, is greater than tolbutamide.⁹ The solubility of metahexamide at a *pH* of 7.0 is equivalent to that of tolbutamide. However, at low levels of *pH* similar to those of the stomach (2.0-3.0) metahexamide, carbutamide and chlorpropamide are all more soluble than tolbutamide.⁹

Since the sodium salt of tolbutamide is much more soluble than tolbutamide, we tested the differences in the rates of absorption of these two compounds. The data in figure 3 show that the absorption of sodium tolbutamide occurs very rapidly in contrast to the rate of absorption of tablets of tolbutamide.

DISCUSSION

These data suggest that the acute potency of carbutamide is approximately one half that of tolbutamide on a milligram for milligram basis. The immediate potency of chlorpropamide under these conditions is similar to that of tolbutamide, as has been shown previously.³ Metahexamide is about three times more potent than tolbutamide under these acute conditions.

On the basis of our previous studies,⁴ we have estimated that the metabolic half lives of tolbutamide, metahexamide, chlorpropamide and carbutamide are

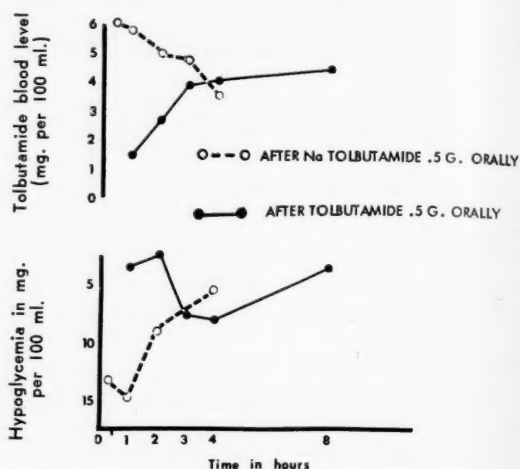


FIG. 3. The comparative blood glucose levels and serum tolbutamide levels are shown after administration of tolbutamide and sodium tolbutamide. Each of the five subjects ingested pulverized tablets of tolbutamide which were mixed thoroughly in warm coffee. On another occasion each of these same subjects ingested sodium tolbutamide in warm coffee. Each plot represents the mean of five values. At twenty-four hours tolbutamide blood levels were negligible and no hypoglycemic effect remained with either preparation.

approximately five hours, nineteen hours, thirty-six hours and thirty-six hours, respectively. It seems likely that the potency of a sulfonylurea during repeated daily administration is a function of its "acute" potency times its metabolic half life. It is, of course, possible that other factors such as differences in distribution, degradation or mechanism of action could also affect the relative clinical potencies but as yet we have been unable to identify any such differences. It would appear from our studies that, immediately after administration, metahexamide is approximately three times more potent than tolbutamide on a milligram for milligram basis. Since the metabolic half life of metahexamide is about four times that of tolbutamide, it would seem reasonable to estimate that when administered daily metahexamide might be about twelve times more potent than tolbutamide on a milligram for milligram basis. This estimate is in agreement with our clinical experience in using the two drugs.

We estimate on the basis of its half life and its immediate potency, that chlorpropamide is six to eight times more potent than tolbutamide. Again, our clinical experience with chlorpropamide has also suggested a potency of this magnitude.

Since carbutamide is about one half as potent as tolbutamide immediately after intravenous injection

and since its half life is about seven times that of tolbutamide, it seems likely that its clinical potency would be three or four times that of tolbutamide on a milligram for milligram basis. Our clinical experience with carbutamide is not adequate to permit an estimation of its potency on clinical grounds. We are not aware of any published studies which show that carbutamide is as much as three times more potent than tolbutamide. However, we do not believe this possibility has been excluded. For example, although the studies of Camerini-Davalos et al.¹¹ suggest that daily doses of carbutamide of 0.5 to 1 gm. are usually adequate in patients who respond to the drug, they reported no experience with doses lower than 0.5 gm. daily. Achelis¹² has observed that, in general, German investigators have not found it necessary to use doses of carbutamide as large as have most American investigators. He believes this fact may account for the low incidence of toxicity (1 per cent) which he observed with carbutamide. It is interesting to speculate what might have happened if the carbutamide tablets which were issued in the United States for clinical trials had been 50- or 100-mg. tablets instead of 500 mg. tablets. It seems probable that lower doses would have been used, and it is conceivable that a lower incidence of toxicity would have resulted.

The importance of establishing clinical potency. If the mechanism of action of each of the active sulfonylureas is the same it does not seem likely that the potency, per se, of a particular derivative would be of critical importance in determining its clinical utility. The usefulness of a sulfonylurea derivative will probably be determined by the toxicity of the drug at the lowest levels of dosage which produce maximal clinical effects. That it may be difficult to establish promptly this critical dosage level on clinical grounds is suggested by the fact that inappropriately large doses of carbutamide, tolbutamide, chlorpropamide, and metahexamide were frequently administered during initial clinical trials with each of these drugs and in some instances many months or even years passed before it became apparent that smaller doses were equally effective.

It seems likely that some of the unfortunate responses to these drugs might not have occurred if more extensive pharmacologic studies in man had been carried out before the large-scale clinical trials. It is possible that some of the toxic reactions to these drugs are not related to the dosage level employed. However, even in reactions of the so-called hypersensitivity type it remains to be established that the incidence and sever-

ity of toxicity are not determined in part by dosage.

It is apparent that carbutamide, chlorpropamide, and metahexamide, when administered for long periods, are far more toxic on a milligram for milligram basis than is tolbutamide. However, these differences are in themselves not necessarily of practical importance since it is the relative toxicities of the sulfonylureas at clinically comparable dosages which are of primary importance. Unfortunately, most of the clinical data concerning relative toxicities of these drugs pertain to cases not receiving comparable doses. For example, the daily dose of tolbutamide which we employ most frequently is 1 gm. (0.5 gm. twice daily). In our experience raising the dose above 1.5 gm. has seldom produced any additional effect. On the basis of our previous observations we believe that 1 gm. of tolbutamide daily is approximately equal to a daily dose of 150 mg. chlorpropamide, 75 mg. of metahexamide, and, on a theoretical basis, as little as 200-400 mg. of carbutamide. If these estimates are approximately correct, it would be of interest to know the relative toxicities of the drugs when these doses are administered. Potentially dangerous liver toxicity occurs occasionally when daily doses exceeding 150 mg. of metahexamide and 300 mg. of chlorpropamide are administered. However, the incidence of toxicity to doses of chlorpropamide of less than 250 mg. daily is not yet established precisely although data on this point should be available soon. Experience has shown that liver toxicity to metahexamide occurs with sufficient frequency, even when small doses are administered, to preclude its use. We are not aware of any published data concerning the toxicity to carbutamide in a large series of patients to whom doses of less than 500 mg. daily have been administered, but this drug has long since been withdrawn from clinical trial in this country.

Absorption rates. It does not appear likely that the differences in the rates of absorption of these drugs have any appreciable effect on their clinical potencies. Indeed, in clinical practice the slow absorption of tolbutamide is an advantage since the drug is excreted rapidly. On the other hand, differences in rates of absorption of sulfonylureas might affect the results of experiments in which relative potencies were estimated by determining hypoglycemic responses immediately after the drugs were administered by mouth. For this reason no conclusion can be reached concerning relative potencies until the rapidity of absorption of each of the drugs tested is known.

In testing the response of a diabetic subject to a single dose of tolbutamide, it would probably be easier

to interpret the results if sodium tolbutamide were used instead of tolbutamide tablets, since absorption of the former is much more rapid and since blood levels of tolbutamide are much higher when comparable doses are administered (see figure 3).

SUMMARY

The potencies of carbutamide, chlorpropamide and metahexamide were determined by comparing the hypoglycemic responses of normal subjects thirty minutes after an intravenous injection of these drugs to the responses of these same subjects to a series of doses of tolbutamide. Under these conditions, carbutamide was about half as potent per milligram as tolbutamide. The potency of chlorpropamide was equal to that of tolbutamide. Metahexamide was approximately three times more potent on a milligram for milligram basis. The hypoglycemia produced by the intravenous injection of two units of glucagon-free insulin was slightly greater than that produced by 250 mg. of tolbutamide.

The absorption of tablets of tolbutamide occurred much more slowly than tablets of chlorpropamide, metahexamide and carbutamide. However, absorption of sodium tolbutamide, which is much more soluble than the parent compound, was very rapid.

On the basis of these observations and our previous findings concerning the metabolism of these drugs theoretical estimates of the clinical potencies of each of these sulfonylureas when administered orally were made. The data suggest that on a milligram for milligram basis, the clinical potency of carbutamide may be expected to be about three to four times that of tolbutamide, although there are no clinical data presently available to prove this statement. Chlorpropamide would be about seven times more potent, and metahexamide twelve times more potent, than tolbutamide.

SUMMARIO IN INTERLINGUA

Le Metabolismo e le Relative Potentias Hypoglycemic de Quatro Sulfonylureas in le Homine

Le potentias de carbutamida, chlorpropamida, e metahexamida esseva determinate per comparar le responsas de normal subjectos trenta minutas post le injection intravenose de un del mentionate drogas con le responsas del mesme subjectos a un serie de doses de tolbutamida. Sub iste conditiones, carbutamida habeva circa un medietate le potentia per milligramma de tolbutamida. Le potentia de chlorpropamida esseva equal a illo de tolbutamida. Metahexamida esseva approximativemente tres vices plus potente super le base de milligramma pro milligramma. Le hypoglycemia producite per le

injection intravenose de duo unitates de insulina libere de glucagon esseva levemente plus grande que le hypoglycemia producite per 250 mg de tolbutamida.

Le absorption de tabletas de tolbutamida se faceva multo plus lentamente que le absorption de tabletas de chlorpropamida, metahexamida, e carbutamida. Tamen, le absorption de tolbutamida a natrium, que es multo plus soluble que le composito matre, esseva rapidissime.

Super le base de iste observationes e nostre previe constataciones relative al metabolismo del drogas sub consideration, estimationes theoric del potentia clinic de cata un del quatro sulfonylureas per via oral esseva elaborate. Le datos pare indicar que a base de milligramma pro milligramma, le potentia de carbutamida es presumemente circa tres a quatro vices illo de tolbutamida, ben que al tempore presente il existe nulle datos clinic que provarea iste estimation. Chlorpropamida esserea circa septe vices plus potente que tolbutamida. In le caso de metahexamida, le relation esserea dece-duo a un.

ACKNOWLEDGMENT

The authors are indebted to Dorothy Antonia Wood, Bob R. Henry, H. Ronald Lee, Lyda L. Long and Vernon Belindo for technical assistance.

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Decreased Response to Intra-arterial Insulin in Acromegaly

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The direct peripheral action of insulin and its fixation in healthy tissues has been demonstrated in both animals¹ and man,²⁻⁴ after injection of the hormone into the brachial or femoral artery. The intra-arterial administration of insulin results in a fall in venous glucose concentration which is much greater in the blood draining the injected than the contralateral limb. An increase in the arteriovenous glucose difference is apparent in the injected limb within five minutes and persists for over an hour. Such effects were observed in the absence of any significant change in the blood flow through skin or muscle,⁴ and hence indicate that a proportion of the insulin administered was immediately fixed in the tissues of the injected arm. The changes in arteriovenous glucose concentration can therefore be used as a measure of the effect of insulin on peripheral glucose uptake in the living subject in various conditions.

In the present study, peripheral responses to insulin in patients with acromegaly and in subjects after an infusion of human growth hormone were compared with those previously observed in healthy adults.

SUBJECTS AND METHODS

Subjects were tested at rest under standard conditions in the laboratory after a fast of six to sixteen hours. Indwelling needles were inserted under local anesthesia into the brachial artery and an antecubital vein of one arm, and into an antecubital vein of the opposite arm. Blood glucose was estimated by a modified Shaffer-Hartman method⁵ in samples taken simultaneously from the three vessels.

Duplicate control samples were taken initially; two units of soluble insulin contained in 2 ml. of isotonic saline were then rapidly injected into the brachial artery and further samples taken at 5, 15, 30, 45 and

60 minutes after the injection.

Ten patients with acromegaly were studied in detail (cases 1 to 10, table 1); less complete data were obtained in three other patients (cases 11 to 13). Two patients (cases 1 and 2) were relatively early examples of the condition with classical features of activity; the disease was apparently progressing slowly in one other patient (case 11), but in the remainder, the advance of the disorder appeared to have been arrested either spontaneously (cases 3 to 6, 9 and 12) or after treatment (cases 7, 8, 10 and 13). None was receiving hormone or other specific therapy at the time of the investigation. One patient (case 10) was a frank though mild diabetic who was not receiving insulin. Glucose tolerance tests had been done at some time during the course of their disease in nine of the other patients; three cases (cases 2 to 4) had normal curves and four (cases 1, 7, 8 and 11) showed some degree of carbohydrate intolerance short of the true diabetic pattern which had been demonstrated in cases 5 and 13. Impairment of insulin sensitivity, as shown by insulin-glucose tolerance tests,⁶ was present in cases 1, 4 and 7 but not in the two other patients so examined (cases 3 and 13).

The effect of human growth hormone on the response to intra-arterial insulin was investigated in three healthy adults. In two, 2 mg. of growth hormone, diluted to a volume of 60 ml., were infused intravenously over thirty minutes. Insulin (two units) was then injected into the brachial artery and samples taken as in previous experiments for a further hour. In the third subject 6 mg. of growth hormone, diluted in 45 ml., were given intravenously over fifteen minutes, and the test dose of insulin was given after a further twenty-five minutes.

Limb blood flow was measured by venous occlusion plethysmography⁷ during two of the infusions of growth hormone and in some of the studies both in normal subjects and in patients with acromegaly.

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TABLE 1

Clinical details of thirteen acromegalic patients

Case No.	Sex	Age	Approximate duration (years)	Clinical activity	Carbohydrate tolerance*	Insulin sensitivity†
1	F	58	2	++	Impaired	Impaired
2	F	27	4	++	Normal	—
3	M	19	9	0	Normal	Normal
4	M	44	30	0	Normal	Impaired
5	M	61	35	0	Diabetic	—
6	M	44	30	0	—	—
7	F	47	10	0	Impaired	Impaired
8	M	48	8	0	Impaired	—
9	M	60	25	0	—	—
10	M	46	15	0	Diabetic	—
11	M	36	15	+	Impaired	—
12	F	33	11	0	—	—
13	F	64	22	0	Diabetic	Normal

*50 gm. glucose.

†Method of Himsworth (1936).

ANALYSIS OF RESULTS

The uptake of glucose at any time, in either forearm, is given by the expression $F(A-V)$:

Where F = blood flow

A = arterial glucose concentration

V = venous glucose concentration

In the initial series of experiments,^{4,8} there was no general or selective change in peripheral blood flow after the intra-arterial injection of two units of insulin. Under standard conditions at rest in the laboratory, forearm blood flow remains remarkably constant for one to two hours.⁹ Since, in the present study, the effect of insulin was assessed by the simultaneous comparison of glucose uptake in both forearms and since random changes in flow were only slight and approximately equal and synchronous on the two sides, the value for F in the expression $F(A-V)$ was considered to be constant. A measure of glucose uptake in either forearm in these circumstances could therefore be obtained by calculation of the arteriovenous glucose difference.

Peripheral glucose uptake is, however, a linear function of the arterial concentration of glucose,¹⁰ and it has been shown that a more accurate indication of the changes in glucose uptake mediated, for example, by insulin, can be obtained by calculating the value of $\frac{A-V}{A}$ (where A = arterial glucose concentration and V = venous glucose concentration), provided that there is no change in blood flow.¹¹

The factors $\frac{A-V_i}{A}$ and $\frac{A-V_c}{A}$ (where V_i = glucose concentration in the vein draining the injected

forearm and V_c = glucose concentration in the vein draining the opposite forearm) may therefore be used as measures of glucose uptake in the injected and in the contralateral forearm respectively.

The value of $\frac{A-V_i}{A}$ however, represents not only the effect of "immediately fixed" insulin, but also that of insulin which was not fixed on its first passage through the capillaries of the injected limb and which has circulated through the lungs and possibly other tissues. The effect of this "circulated" insulin is indicated by the changes in glucose uptake in the contralateral, noninjected forearm and can be assessed by the factor $\frac{A-V_c}{A}$. The effect of the "immediately fixed" insulin on glucose uptake can thus be calculated from the expression:

$$\left(\frac{A-V_i}{A} \right) - \left(\frac{A-V_c}{A} \right)$$

i.e. $\left(\frac{V_c-V_i}{A} \right)$

The results of detailed studies in thirteen healthy adults⁴ were analyzed in this way, and compared with the values obtained in the present study of patients with acromegaly.

RESULTS

1. Response to intra-arterial insulin in acromegaly.

The mean pattern of response to intra-arterial insulin in euglycemic acromegalics, i.e., those patients whose fasting blood sugar was less than 100 mg. per ml. (cases 1 to 8, tables 1 and 2) differed from that

TABLE 2
Effect of intra-arterial insulin on blood sugar (mg. per 100 ml.) of eight euglycemic acromegalics

Case No.	Time (minutes after insulin)	Arterial (A)						Ipsilateral Venous (Vi)						Contralateral Venous (Vc)					
		0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60
1		90	92	83	83	89	82	94	92	65	76	88	85	91	91	73	81	84	90
2		92	89	67	74	90	70	76	71	38	41	57	—	87	76	61	57	74	74
3		80	75	63	57	77	78	72	68	64	57	78	76	80	72	62	63	69	82
4		100	93	79	80	87	92	93	74	83	83	83	82	87	83	81	83	82	80
5		94	97	81	78	80	77	99	83	83	80	75	66	98	89	80	72	72	73
6		93	90	81	85	88	83	90	75	56	61	63	73	88	86	78	70	75	73
7		94	91	87	90	90	89	87	74	71	49	70	80	89	88	83	78	81	81
8		90	88	69	76	90	80	88	75	72	76	83	80	84	87	70	81	78	86
Mean		92	89	76	78	86	81	87	76	67	65	75	77	88	84	73	73	77	80
Mean levels in thirteen normal subjects		84	81	56	60	75	80	77	65	37	35	51	59	75	72	46	44	59	69

observed in healthy adults (table 2 and figure 1). After the injection of two units of insulin into the brachial artery, the arteriovenous glucose difference in the ipsilateral arm was much less in acromegalic subjects than in healthy adults. Similarly, there was a smaller fall in arterial glucose concentration and in the venous glucose concentration of the opposite arm. The mean effect of "immediately fixed" insulin, calculated from the expression $\frac{V_c - V_i}{A}$, is shown in figure 2. In healthy

subjects there was a significant rise in uptake after the injection of insulin, at 5, 15, 30 and 60 minutes.*

In acromegalic patients, however, a significant increase in uptake was only recorded at five minutes ($t = 2.466$, $P < 0.05$) and even this increase was significantly less than that observed in the control group

* ($t = 4.329$, $P < 0.001$ at five minutes; $t = 2.678$, $P < 0.05$ at fifteen minutes; $t = 2.911$, $P < 0.05$ at thirty minutes; $t = 4.571$, $P < 0.001$ at sixty minutes).

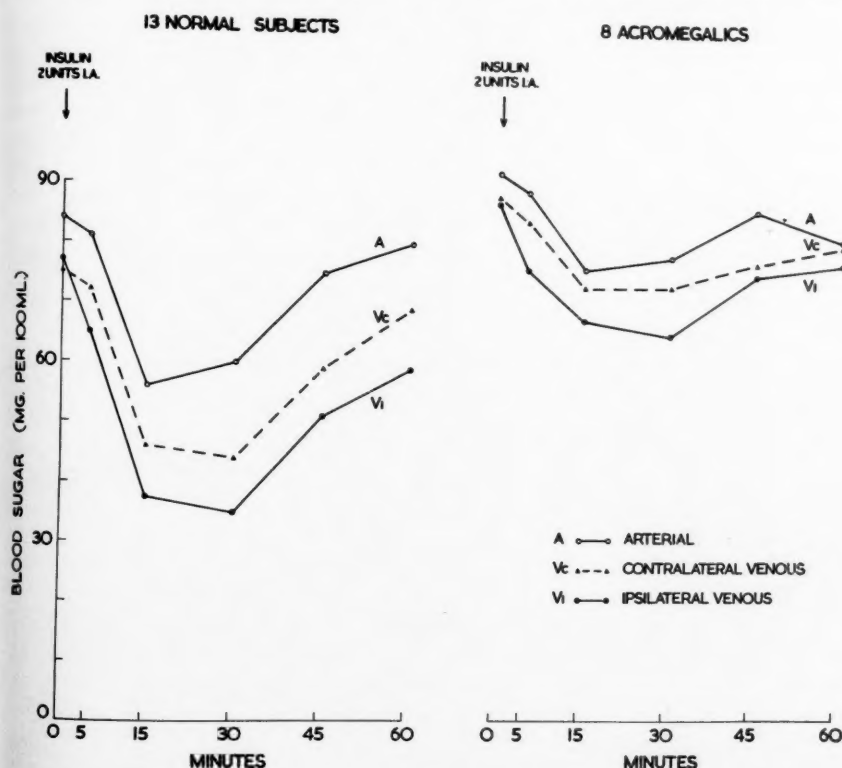


FIG. 1. Mean blood sugar levels after intra-arterial injection of two units of insulin in (a) thirteen normal subjects and (b) eight euglycemic acromegalics.

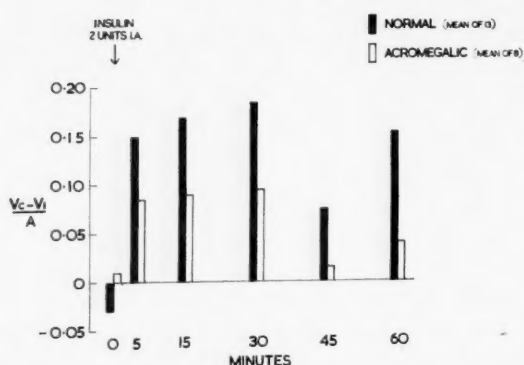


FIG. 2. Effect of "immediately-fixed" insulin ($\frac{V_c - V_i}{A}$) in normal and acromegalic subjects.

($t = 12.8$, $P < 0.001$). Thus, in acromegalic subjects, there appears to be an impairment of the action of "immediately fixed" insulin.

The changes in glucose uptake which occur in the contralateral arm are illustrated in figure 3. Significant differences between the mean response in acromegalic subjects and healthy individuals were recorded at 15, 30, 45 and 60 minutes.*

The mean effect of intra-arterial insulin was undoubtedly reduced in patients with acromegaly, and some impairment of the response was apparent in nine (70 per cent) of the thirteen subjects. Four (cases 2, 6, 9 and 12), however, showed a pattern of response resembling that observed in the control group. There was no relation between individual responses to insulin

* ($t = 2.264$, $P < 0.05$ at fifteen minutes; $t = 2.105$, $P < 0.05$ at thirty minutes; $t = 2.332$, $P < 0.05$ at forty-five minutes; $t = 2.245$, $P < 0.05$ at sixty minutes.

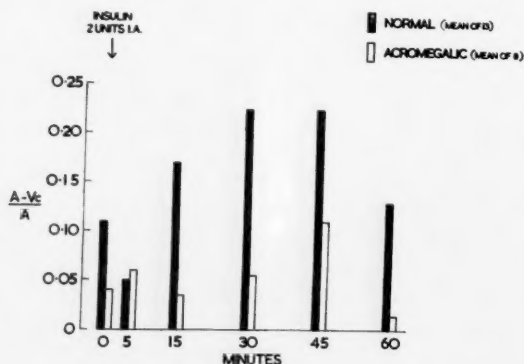


FIG. 3. Effect of "circulated" insulin ($\frac{A - V_c}{A}$) in normal and acromegalic subjects.

and the activity, duration or degree of the disorder, the age or sex of the patient, or the response to the glucose tolerance test.

2. Response to intra-arterial insulin in normal subjects after infusion of human growth hormone.

In the subject who received an infusion of 6 mg. human growth hormone twenty-five minutes before the test dose of insulin, the response was reduced compared with that seen in the control study (figure 4). This modified response was similar in pattern to that seen in the majority of the acromegalics. In one of the subjects given 2 mg. of growth hormone, the peripheral response to insulin was less than that seen normally, while the remaining subject showed no impairment of response. There was no change in blood flow during or after the infusion of 2 mg. of growth hormone.

DISCUSSION

The method of analysis in the present study is based on the fact that when insulin is given into a peripheral artery, its action is, in the first instance, confined to the tissues supplied by that vascular bed. A widening of the arteriovenous glucose difference in the injected limb after the intra-arterial administration of insulin was first demonstrated in rabbits and depancreatized dogs.^{1,12} Similarly, when insulin was injected into the femoral artery of healthy men, the fall in glucose concentration was much greater in venous blood draining the injected leg than on the opposite side.² The direct peripheral effect of insulin was subsequently studied in more detail in the human forearm.⁴ After the intra-arterial injection of two units of insulin, there was a rapid fall in glucose concentration of venous blood from the injected forearm, which was sustained for at least two hours. Changes in venous glucose concentration in the opposite forearm were slight and only temporary. These findings were interpreted as evidence of the immediate fixation of insulin in healthy tissues. The insulin fixed in this way during its first passage through the capillaries of the injected arm avoids the risk of modification or inactivation by visceral mechanisms. Moreover, the time during which the hormone is exposed to any modifying influences in the plasma is reduced to a minimum.

The immediate localization of insulin in significant amounts in the tissues *in vivo* is not unexpected when previous *in vitro* studies are considered. Stadie and his colleagues¹³ showed that exposure of isolated rat diaphragm to an insulin-containing medium for as short a time as ten seconds led to increased glycogen synthesis; they subsequently demonstrated that insulin was irreversibly fixed in a similar way by other tissues.¹⁴

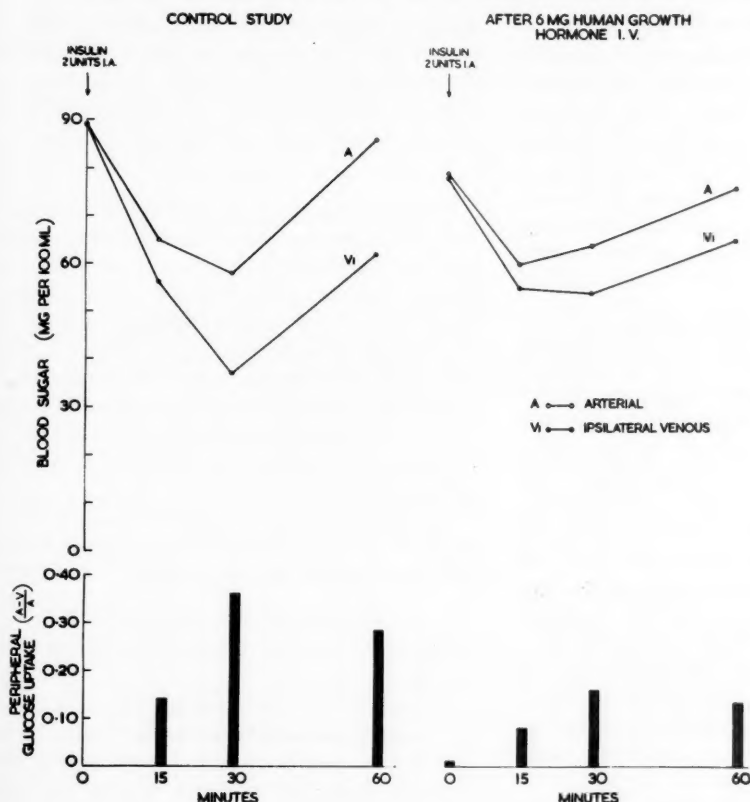


FIG. 4. Response to intra-arterial insulin in a healthy subject. Comparison of (a) control study with (b) study twenty-five minutes after an intravenous infusion of 6 mg. of human growth hormone.

The present findings suggest that there may be two separate types of abnormality of insulin activity in acromegaly. Firstly, there is an impairment of the immediate action of the hormone during its initial passage through the tissues of the injected arm. This reduction in the effect of "immediately fixed" insulin might be due either to interference with the normal, instantaneous fixation in peripheral tissues, or to inhibition of peripheral action after fixation has taken place. A further possibility is that there is a qualitative change in peripheral glucose metabolism in this condition.

Secondly, there would seem to be impairment of the peripheral response to the insulin which has circulated through the lungs and possibly other viscera. Although this abnormality may be due to a mechanism identical with that causing the altered response to "immediately fixed" insulin, the degree of impairment of peripheral glucose uptake appears to be greater in the case of "circulated" insulin. In particular, two acromegalics (cases 7 and 10) showed a definite response to insulin in the injected limb but only a very slight effect in the

contralateral limb. This may mean that, in addition to the disturbance of insulin action in the peripheral tissues, there is some other site of antagonism in the plasma or in the lungs or other viscera. The "immediately fixed" insulin may be less susceptible to inactivation in the plasma because of the very short period of exposure before local fixation in the tissues.

Wright,¹⁵ noting a discrepancy between the plasma insulin levels in normal subjects as recorded by himself and by Vallance-Owen and Hurlock¹⁶ and the higher levels recorded by a technic involving dilution of the plasma,¹⁷ suggested that dilution might liberate insulin from some inactive form or might inactivate a circulating insulin antagonist. The discrepancy between results obtained by the two methods is much greater with plasma from acromegalic patients^{16,18} suggesting the presence of a correspondingly greater concentration of the insulin antagonist or inactivator. Insulin inhibitors associated with various fractions of the serum proteins have also been found in the plasma of diabetics who have never been treated with insulin.^{19,20} In healthy

adults, insulin antagonists have been demonstrated in association with plasma albumin²¹ and α -2 globulin fractions.²² These factors are not found after hypophysectomy^{23,24} but reappear after the administration of growth hormone.²⁵ These various anti-insulin factors, although humoral in origin, probably act at the periphery and not directly in the circulation. However, Marsh and Haugaard²⁶ have demonstrated an insulin-neutralizing substance in the serum of normal and of diabetic subjects which is apparently effective in the bloodstream.

The diabetogenic effect of anterior pituitary extracts and of growth hormone is well established²⁶⁻²⁸ although diabetes mellitus is not an inevitable complication of acromegaly, the reported incidence being less than 30 per cent.^{29,30} A much higher incidence of disturbed carbohydrate metabolism was, however, apparent in the present series, an impairment of insulin action at the periphery being revealed in 70 per cent of the acromegalics. It is interesting to note that such impairment was often demonstrated in the absence of clinical or biochemical evidence of diabetes. It is possible that this may reflect the functional integrity of the pancreatic islets, since diabetes is more likely to occur in those acromegalics who have a familial predisposition to the former disease.²⁹ It may also be relevant that a similar abnormality of the response to insulin has been observed in nondiabetic subjects during prolonged treatment with adrenocortical steroids.³¹

The finding that previous administration of human growth hormone can apparently reproduce this abnormality of peripheral response in healthy adults is of considerable interest and supports the suggestion that resistance to insulin in acromegaly is dependent on excess growth hormone. In the present experiments, however, there was no correlation between impairment of the response to insulin and clinical activity of the disease. Moreover, although crude preparations of the anterior pituitary inhibit glucose uptake in the isolated rat diaphragm, whether injected in vivo or applied in vitro during incubation, purified growth hormone inhibits glucose uptake only when injected in vivo and at least three hours before sacrifice.^{32,33} It would appear, therefore, that although growth hormone is not a direct inhibitor of peripheral glucose uptake, it may alter in some way the metabolic activity of the tissues. Since the response to insulin was impaired in the absence of other evidence of activity of the acromegalic process, it is possible that this metabolic abnormality, once induced, is permanent, the situation being analogous to that occurring in the skeleton. The precise relationship between these physical and biochemical changes and

the concentration or activity of growth hormone is, however, still not clear. In any case, it seems likely that the abnormality of carbohydrate metabolism in acromegaly is complex, and does not bear a simple relationship to excessive production of growth hormone.

SUMMARY

Peripheral glucose uptake was measured in the forearm of healthy adults and of patients with acromegaly, after the intra-arterial injection of two units soluble insulin. Peripheral glucose uptake was significantly reduced in 70 per cent of the acromegalic subjects. There was no correlation between the reduction in response to insulin and clinical activity of the disease.

Peripheral glucose uptake was reduced in normal subjects by the previous administration of human growth hormone, in a manner comparable with that seen in acromegaly.

The mechanism of antagonism to insulin in acromegaly is discussed and it is suggested that both tissue and serum factors may be involved.

SUMMARIO IN INTERLINGUA

Reducite Responsa a Insulina Intra-Arterial in Acromegalia

Le captation peripheric de glucosa esseva mesurate in le antebraccio de adultos normal e de patientes con acromegalia, post le injection intra-arterial de duo unitates de insulina de forma solubile. Le captation peripheric de glucosa esseva reduce significativamente in 70 pro cento del subjectos acromegalic. Non esseva constatate un correlation inter le reduction del responsa a insulina e le activitate clinic del morbo.

Le captation peripheric de glucosa esseva reduce in subjectos normal per le previe administration de hormon de crescentia human. Iste reduction esseva simile a illo notate in acromegalia.

Es discute le question del mecanismo del antagonismo contra insulina in acromegalia. Es presentate le conception que le factores responsabile es de character tanto tissular como etiam seral.

ACKNOWLEDGMENT

We are grateful to Dr. R. F. Hellon for assistance in the statistical analyses and to Professor Russell Fraser for his help in obtaining human growth hormone. Certain expenses were defrayed by a grant from the Endowment Funds of St. Thomas's Hospital.

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A partial explanation for the failure to find an increase in total corticosteroid excretion in the face of high plasma levels was provided by C. J. Migeon, J. Bertrand and B. E. Wall (*J. Clin. Invest.* 36: 1350, 1957). These investigators demonstrated that the rate of disappearance of cortisol-4-C-14 from the blood was greatly delayed in pregnancy. This observation was subsequently confirmed by M. Cohen, M. Stiefel, W. J. Reddy and J. C. Laidlaw (*J. Clin. Endocrinol. & Metab.* 18:1076, 1958). The additional observation has been made by N. P. Christy, E. Z. Wallace, W. E. L. Gordon, and J. W. Jailer (*J. Clin. Invest.* 38:299, 1959) that tetrahydrocortisol as well as cortisol has a prolonged half

life in the blood of pregnant women. The impaired metabolism of cortisol differs from the impairment which occurs in cirrhosis of the liver, in which the metabolism of the reduced hormone is apparently unaffected.

Although the reported decrease in the rate of metabolism of cortisol helps to explain the pattern of the urinary excretion of corticosteroids, it fails to account for the elevation of the plasma level of cortisol and the absence of signs of hyperadrenalcorticism which would be expected with elevations of the plasma cortisol level.

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Studies of Abnormal Glucose Metabolism in Pregnancy

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Any investigation of ostensibly healthy women who have glucosuria in pregnancy may reveal either a decrease in the renal threshold for glucose or a decreased tolerance for glucose, or both. Pregnant women with impaired glucose tolerance have been termed prediabetic or potential diabetics. Complications of pregnancy, such as abortion, premature delivery, stillbirth, neonatal death, congenital abnormalities or excessively large infants, are known to be associated with impaired tolerance to a degree approaching that seen in diabetic pregnant women.¹⁻⁴ The underlying cause of these abnormalities is not known. The individual and interdependent effects of pituitary, adrenal, thyroid and gonadal hormones in pregnancy and their relationship to maternal carbohydrate and lipid metabolism and to fetal development has not been clearly defined.

The present study was undertaken in selected pregnant women:

1. to determine the significance of impaired oral as compared to impaired intravenous glucose tolerance on the outcome of pregnancy, and
2. to determine if there were associated or relevant differences in lipid metabolism, thyroid function, and serum insulin-like activity in those women with impaired tolerance as compared to those with normal tolerance.

METHOD OF STUDY

A. Selection of subjects

One hundred and twenty-eight pregnant women without gross obesity or evidence of intercurrent or chronic disease were selected from the clinic populations of the Mary Fletcher Hospital and the DeGoesbriand Memorial Hospital, from a home for unmarried mothers, and by referral from practicing obstetricians. The criteria for selection were as follows:

1. Glucosuria in current or past pregnancies
2. Family history of diabetes mellitus

Presented at the Twentieth Annual Meeting of the American Diabetes Association in Miami Beach on June 11, 1960.

From the Metabolic Unit of the Department of Medicine, College of Medicine, University of Vermont, Burlington, Vermont.

3. Past history of abortion, stillbirths, fetal abnormalities and/or maternal complications

4. Past history of excessive-sized infants at birth ($> 4,000$ gm.)

5. Symptoms of hypoglycemia three to five hours postprandially.

B. Testing of subjects

Prior to tolerance tests, all subjects were prescribed a high-carbohydrate intake (> 250 gm.) for three days.

Standard oral glucose tolerance tests, using a test dose of 100 gm. of dextrose in flavored solution, were given to 119 subjects. Venous blood samples were analyzed for glucose before, and at 30, 60, 120, and 180 minutes after the oral glucose. The Somogyi modification of the Shaffer-Hartman method⁵ or the Auto-Analyzer was used for glucose determinations. Urinary glucose was estimated during each time period, using glucose oxidase reagents.

Thirty-one subjects, twenty-two of whom had had previous oral tolerance tests, were given a rapid intravenous glucose tolerance test (modification of the Amatuzio method)⁶ in which 25 gm. of 50 per cent glucose is injected over a two- to four-minute period and venous blood samples are taken four to five minutes after the injection and at 10, 20, 30, 45, and 60 minutes thereafter. The excess of blood glucose over the fasting value is plotted semilogarithmically against time, and the rate of disappearance of excess glucose from the circulation calculated from the formula $k = \frac{0.693}{t}$, where t = time in minutes for the half life of the excess glucose. The result is expressed as the percentage of excess glucose disappearing from the circulation per minute. (In Amatuzio's series, the glucose disappearance rate for normals varied from 3.0 to 4.8 per cent, and for mild diabetics 0.93 to 2.46 per cent per minute.)

Other serum specimens were obtained at the same time intervals and immediately frozen and stored at -4° C. for later lipid analyses and hormone assays.

C. Criteria for abnormality of tolerance tests

1. Oral. The criteria suggested by Wilkerson and Remein⁷ were employed. Two or more values had to

equal or exceed the following: 110 mg. per cent FBS, 170 mg. per cent at 60, 120 mg. per cent at 120, and 110 mg. per cent at 180 minutes.

2. Intravenous. To be considered abnormal the excess glucose disappearance rate had to be less than 3 per cent per minute.

D. *In vitro* assay for insulin-like activity

Selected serum specimens from normal and abnormal oral and intravenous tolerance tests were assayed for insulin-like activity in the fasting state and after glucose stimulation. With minor modifications the method described by Beigelman,⁸ estimating glucose uptake by the rat epididymal fat pad, was used. Known concentrations of purified insulin* between 10 and 1,000 μ U per milliliter have given satisfactory and reproducible dose response curves. Pooled human serum standards have been found to contain approximately the same amount of insulin-like activity when assayed repetitively. The iodometric method⁹ has been used for estimating the glucose content of the incubation medium.

E. Serum cholesterol

Serum specimens from fasting pregnant women in their third trimester were analyzed for total cholesterol and cholesterol esters by the method of Zak et al.¹⁰

F. Serum protein-bound iodine

PBI determinations were made on the sera of certain pregnant women, using the method of Zak et al.¹⁰

RESULTS

A. Oral glucose tolerance tests

Of the 128 patients given glucose tolerance tests, sixty-six had normal glucose tolerance and sixty-two had impaired tolerance. Table 1 illustrates that thirty-nine of those women with normal tolerance showed glucosuria at blood glucose concentrations of less than 140 mg. per 100 ml., and twenty-seven did not have glucosuria. There was no significant difference in the average age, parity, or the number of patients who had had previous abortions, large babies, or fetal loss. The incidence of family history of diabetes was about 50 per cent in both groups. Hypoglycemic symptoms were rare. The only attribute that distinguished the two groups from one another was the more frequent incidence of random glucosuria in those subjects with the decreased renal threshold. For practical purposes, therefore, those with renal glucosuria of pregnancy need not be categorized apart from those others with normal glucose tolerance.

Table 2 compares the vital statistics and past histories

*Kindly supplied by Dr. Otto K. Behrens of Eli Lilly and Company.

TABLE 1
Sixty-six normal glucose tolerance tests with and without glucosuria (See text)

	Normal	Normal with renal glucosuria
Number of subjects	27	39
Average age	24.1	24.6
Average parity	2.2	1.5
History of previous abortion	6	7
History of previous perinatal death	1	1
History of overweight infants	3	1
Family history of diabetes	14	19
Random glucosuria	8	34
Hypoglycemic symptoms	1	2

of the entire normal tolerance group of sixty-six (including those with renal glucosuria) with those sixty-two subjects who had abnormal tolerance. Here again the age group was comparable, although the average parity was one greater in those with abnormal tolerance. There were twenty-nine abortions in nineteen subjects with impaired tolerance, which is not significantly more numerous than the twenty-three abortions in thirteen women with normal tolerance. Seven subjects with abnormal tolerance had had twelve previous fetal deaths, whereas only two subjects with normal tolerance had had two fetal deaths. There were twenty overweight babies in nine women with abnormal tolerance, and only five in four women with normal tolerance. Family history of diabetes was less prevalent in those women with abnormal tolerance. Hypoglycemic symptoms were more prevalent, but the presence of a low blood sugar was verified in only three subjects.

Table 3 lists the results for the current pregnancy. Most of the tests were performed in the third trimester; those showing abnormal glucose tolerance were done about five weeks later than those showing normal tolerance. All infants were full-term; none of the births was induced. Although the average birth weights for each group were not significantly different, there were ten infants in excess of 4,000 gm. weight in the im-

TABLE 2
Comparison of sixty-six normal and sixty-two abnormal tolerance tests (See text)

	Normal (including renal glucosuria)	Abnormal
Number of subjects	66	62
Average age	24.4	25.9
Average parity	1.8	2.8
History of previous abortion	13	19
History of previous perinatal death	2	7
History of overweight infants	4	9
Family history of diabetes	33	20
Random glucosuria	42	54
Hypoglycemic symptoms	3	10

paired tolerance group, compared to three in the normal tolerance group. There were five instances of fetal loss (by abortion or perinatal death) in the impaired tolerance group, and none in the normal tolerance group. None of these women having overweight infants or perinatal death had been treated with diet or insulin. While this series is too small for statistical significance, the above results are suggestive of the unphysiological consequence of impaired glucose tolerance.

TABLE 3
Present pregnancy—glucose tolerance tests

	Normal (including renal glucosuric)	Abnor- mal
Number of subjects	66	62
Duration of pregnancy when tested	27	32
Average birth weight	3,303	3,527
Overweight infants	3	10
Abortion	0	1
Perinatal death	0	4
Maternal complications	0	1

B. Intravenous glucose tolerance tests

Twenty-seven of the sixty-two women with impaired oral glucose tolerance were then tested by the intravenous method described above. Table 4 illustrates that nineteen of these women had normal rates of glucose removal (> 3 per cent per minute). There were eighteen normal living infants and one overweight infant produced from these women. In contrast, eight of the women had abnormal intravenous as well as abnormal oral tolerance. There were three perinatal deaths and two overweight infants in five of these women, none of whom had been treated with diet or insulin. One woman had hypercholesterolemia (377 mg. per cent) and suffered a cerebrovascular accident. She did not abort, and on recovery was treated with insulin and diet (see below) and delivered a normal infant at term. The remaining two women had also been treated with diet and delivered normal infants. The abnormality in the intravenous glucose tolerance test was always mild; the rate of excess glucose removal was in the order of 2.4 to 2.8 per cent per minute, which is in the intermediate zone of Amatozuo's original series between mild diabetes and normal range.⁹

C. Serum cholesterol

It has previously been reported that the serum cholesterol and other lipid fractions rise during pregnancy.¹¹ The reason for this has not been established. Since one of our subjects had a cerebrovascular accident and was found to have hypercholesterolemia as well as abnormal

TABLE 4
Pregnancy—intravenous glucose tolerance tests

	Normal	Abnormal
Number of subjects	19	8
Normal infants	18	3*
Overweight infants	1	2
Perinatal deaths	0	3
Maternal complications	0	1†

*Treated.

†Treated; delivered normal infant.

oral and intravenous glucose tolerance, the serums of other patients were analyzed for cholesterol. Fifteen subjects who had normal glucose tolerance had an average serum cholesterol concentration of 257 mg. per cent (S.D. ± 20), while fifteen subjects who had abnormal oral, and/or intravenous tolerance, had an average of 322 mg. per cent (S.D. ± 39). ($p < 0.01$.) The esterification of cholesterol was 75 per cent in women with normal tolerance and 71 per cent in women with impaired glucose tolerance.

D. Protein-bound iodide

Serum protein-bound iodide is generally increased in pregnancy, due to an increase of the iodine-binding globulin, so that the range of normal for pregnancy is higher than for nonpregnant euthyroid women. In this study twenty-three women were screened to detect gross elevations in PBI beyond the usual increased range for pregnancy. Twelve women with normal glucose tolerance had an average PBI of 6.4 μ g. per cent, while eleven women with impaired tolerance had an average PBI of 7.3 μ g. per cent. The difference is obviously not significant.

E. Estimation of serum insulin-like activity

Serum insulin-like activity while fasting and after intravenous glucose was compared in two nonpregnant women, eight women with normal glucose tolerance, and six with abnormal tolerance. The results are shown in table 5. The fasting concentrations of insulin-like activity varied greatly, as did the response to glucose, in all three groups. Fasting insulin-like activity was as high or higher in the normal pregnant than in the nonpregnant women, and was still higher in the women with abnormal tolerance. Following intravenous glucose there was always a rise in insulin-like activity within ten to fifteen minutes after injection. This rise was two- to fourfold in the nonpregnant and pregnant women with normal tolerance, except for instances of a twenty- and a fortyfold rise in the latter group. In those women with abnormal tolerance, the ten- to fifteen-minute postglucose rise was 1.3 to four times the fasting value. Although the table suggests slightly increased and prolonged elevations of insulin-like activity in the women

TABLE 5

Range of insulin-like activity (in microunits/milliliter) in serum diluted 1:1, during intravenous glucose tolerance test (estimated by glucose uptake in epididymal fat pad)

	Non-pregnant women	Pregnant women Normal tolerance	Pregnant women Abnormal tolerance
Fasting	24- 91(2)	<10-312(8)	118-1,225(6)
10-15'	47-358(2)	188-551(6)	1,342-1,600(2)
20-30'	—	80-258(6)	85- 830(6)
45-60'	16- 61(2)	<10-166(8)	43- 329(5)

with abnormal tolerance, these data are too few and too variable to be significant.

TREATMENT

1. *Diet.* Two standard diets were used to treat selected patients who had an abnormal glucose tolerance. Each diet contained approximately 165 gm. of carbohydrate, and 100 gm. of protein. The fat content was varied between 60 and 90 gm. by changing the amount of skim or whole milk used. Total calories were therefore between 1,600 and 1,975. The caloric prescription was varied according to the stature and activity of the patient, and whether or not there had been a tendency to gain excessive weight during pregnancy.

2. *Insulin.* In certain patients, particularly those with abnormal intravenous tolerance tests, NPH insulin was prescribed in doses of 10 to 15 U before breakfast each day. The dose was adjusted by urine tests and occasional postprandial blood glucose estimations.

3. *Results.* A total of fifteen women were treated with diet, or diet plus insulin. Twelve of the fifteen received diet therapy alone, while three received diet and insulin. Three of the fifteen had had abnormal oral and intravenous glucose tolerance; the outcome of pregnancy was favorable in each instance. The remaining twelve women had an abnormal oral tolerance only; seven of these had a normal intravenous tolerance, and eight had not been tested by the intravenous method. In this small group of fifteen so treated there were no overweight babies produced and there were no perinatal deaths or other fetal complications. It should be noted, however, that there were thirty-eight women with abnormal oral tolerance who were not treated either with diet or with insulin. Of these thirty-eight there were six who had overweight infants and two other perinatal deaths; none had been treated. Unfortunately, it is not known whether these women also had had abnormal intravenous tolerance. Of the remaining thirty who had normal infants, seven had been treated with diet, one with diet and insulin, and twenty-two had received no therapy.

DISCUSSION AND CONCLUSIONS

No attempt has been made in this study to determine statistically the incidence of abnormal carbohydrate tolerance in the average obstetrical population, or its significance in regard to fetal or maternal complications of pregnancy or to the incidence of diabetes mellitus in the mother or child. The number of subjects is too small for statistical significance, yet the results substantiate the results of others¹⁻⁴ who have shown that the incidence of overweight infants and perinatal death is greater in those women with impaired glucose metabolism. However, in this present study there was a large number of women with abnormal oral glucose tolerance whose outcome of pregnancy was entirely favorable. In the majority of these women, as noted above, there was a normal tolerance to intravenous glucose, implying that their insulin response and homeostatic mechanisms were adequate, provided the stimulus is sufficient. Others, however, showed slight retardation in the removal rate of intravenously administered glucose, suggesting greater impairment of glucose utilization. These women with impaired intravenous tolerance had more overweight infants and suffered greater fetal loss than those women with abnormal oral but normal intravenous tolerance.

The results of the assay of insulin-like activity, although not conclusive, suggest that pregnant women may secrete normal or greater than normal amounts of insulin in response to glucose stimulation. This in turn suggests that there are other factors which are hyperglycemic in their effect, either through increased gluconeogenesis, or by insulin inactivation or by blocking the effect of insulin. The nature of these factors has not been learned from these studies. However, the abnormal concentrations of serum cholesterol in the subjects with abnormal glucose tolerance suggest that other hormones, either of pituitary, adrenal, or placental origin may be causing abnormalities in lipid as well as carbohydrate metabolism, with consequent deleterious effects on the outcome of pregnancy.

The effect of diet or insulin treatment in this small series was attended by favorable outcomes of pregnancy, but again no statistical significance can be stated.

SUMMARY

One hundred and twenty-eight pregnant women, considered potential diabetics because of glucosuria, symptoms of hypoglycemia, family history of diabetes, or a past history of overweight infants, perinatal deaths, or abortions, were given oral and/or intravenous glucose tolerance tests. Sixty-six had normal tolerance (including thirty-nine with renal glucosuria) and sixty-two had impaired tolerance.

Past history of overweight infants and fetal loss was more prevalent in women with impaired tolerance; family history of diabetes was less frequent.

In the current pregnancy, overweight infants and fetal loss were also greater in the women with abnormal oral tolerance and greatest in those women who also had impaired intravenous tolerance. Serum cholesterol concentration was significantly higher in women with abnormal tolerance compared to those with normal tolerance. Serum protein-bound iodine concentration was not significantly different in the two groups. Serum insulin-like activity was present in both groups, equal to or greater than in nonpregnant controls, and increased following glucose stimulation, with no significant difference between the normal and abnormal groups.

Twelve women with abnormal oral tolerance were treated with diet and three with abnormal oral and intravenous tolerance with diet and insulin; the outcome of pregnancy was favorable in all of these.

SUMMARY IN INTERLINGUA

Anormal Metabolismo de Glucosa in Pregnantias

Cento vinti-otto gravidas—considerate como potencialmente diabetic a causa de glucosuria, symptommas de hypoglycemia, anamnese familial de diabete, o le antecedente de parturition de infantes a pesos excessive, de morte perinatal del infante, o de abortos—esseva subicite a oral e/o intravenose tests de tolerantia pro glucosa. Sexanta-sex monstrava un tolerantia normal—incluse trenta-novem con glucosuria renal—e sexanta-duo habeva un tolerantia defective.

Le antecedente de infantes a pesos excessive o de perditas fetal esseva plus prevalente in feminas con tolerantia defective. Le anamnese familial de diabete esseva minus frequente in illas.

In le pregnantia currente, infantes a excessos de peso e perdita fetal esseva etiam plus frequente inter feminas con anormalitate del tolerantia oral e attingeva le plus alte incidentia inter illas qui etiam habeva un defectiva tolerantia intravenose.

Le concentration de cholesterol sereal esseva significativamente plus alte in feminas con anormalitate del tolerantia in comparison con feminas in qui le tolerantia esseva normal. Le concentration de iodo ligate a proteina in le sero non differeva significativamente inter le duo gruppos. Activitate insulinoide esseva presente in le seros de ambe gruppos. Su valor esseva equal o superior a illo in non-pregnante feminas de controlo. Illo cresceva post stimulation per glucosa, sin ulle differentia significative inter le gruppo normal e le gruppo anormal.

Dece-duo feminas con anormalitate del tolerantia oral esseva tractate con mesuras dietari; tres con anormalitate del tolerantia oral e intravenose esseva tractate con mesuras dietari e insulina. In omne istas, le termination del pregnantia esseva favorabile.

ACKNOWLEDGMENT

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Electron Microscopy of Glycogenic Changes in Beta Cells in Experimental Diabetes

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INTRODUCTION

The discovery by Toreson¹ that hydropic changes in the beta cells of the islets of Langerhans and in the exocrine ductular epithelium are actually caused by abnormal accumulation of glycogen was a contribution of major importance in establishing the relationship of the pathologic changes in the pancreas to the disturbance of carbohydrate metabolism in diabetes mellitus. The demonstration of glycogen within beta cells has stimulated a continuing interest both in the mechanisms involved in its deposition and the effects, if any, it has on these cells.

"Hydropic" changes in the islets of Langerhans were first described by Weichselbaum and Stangl² in 1901 in pancreases from comatose diabetic patients. Subsequently, Allen³ produced similar lesions in the islets and ducts of dogs made diabetic by partial pancreatectomy. Homans^{4,5} likewise observed degranulation and hydropic changes in partially pancreatectomized cats and dogs and noted that they were confined to beta cells. Similar hydropic changes have been reported in naturally occurring diabetes in various laboratory animals. Since none of these investigators was able to demonstrate fat, mucin or glycogen the vacuoles were considered to be of an aqueous nature.

As indicated above, it was not until 1951 that the true nature of hydropic degeneration was elucidated by Toreson¹ who demonstrated that "hydropic" changes were produced by the autolytic removal of intracytoplasmic accumulations of glycogen prior to fixation rather than to the presence of excessive quantities of water or fluid. In more recent years Lazarus and Volk⁶⁻⁹ have performed extensive studies of glycogenic infiltrations in

both beta cells and ductular epithelium in a variety of experimental diabetic states.

Experimental glycogen infiltration in the islets of Langerhans. Small deposits of glycogen have been demonstrated histochemically in pancreatic ductular epithelium of normal human embryos¹⁰ and normal adult dogs.¹¹

Abnormal accumulations of glycogen in the islets and ductular epithelium have been produced in a number of laboratory animals by a variety of technics. Partial pancreatectomy has been found to produce diabetes with changes described as hydropic in the remaining islets of both dogs and cats.⁸⁻⁶ More recently Bencosme, Mariz and Frei¹² have demonstrated that these hydropic changes in ductular epithelium and islets of partially pancreatectomized diabetic dogs are due to the presence of glycogen. Although removal of 99.5 per cent of the rat pancreas leads to the rapid development of severe diabetes,¹³ glycogenic accumulations in the pancreatic remnants of surviving treated animals have not been described.

In 1943, Dunn, Sheehan and McLetchie¹⁴ first described the cytotoxic effects of alloxan on the beta cells of rabbits. Although alloxan diabetes can be produced in a variety of experimental animals, glycogenic infiltration in the remaining beta cells has been demonstrated only in certain species. Vacuolation of ductular epithelium has been described in alloxan diabetic dogs,¹⁴ and glycogen has been demonstrated in both ductular epithelium and beta cells of diabetic rabbits.¹⁵

Dohan and Lukens¹⁶ described hydropic changes in the islets of cats by giving repeated intraperitoneal injections of glucose. In a few instances permanent diabetes was produced. These hydropic changes have been shown by Theodossiou¹⁷ to represent abnormal accumulation of glycogen.

More recently, purified hormonal preparations (e.g., growth hormone⁸ and glucocorticosteroids)^{7,8,18,19} have been shown to be diabetogenic and result in abnormal accumulation of glycogen in a number of laboratory animals.

All of the above methods for producing diabetes induce a relative deficiency of insulin resulting in the

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development of hyperglycemia. Since release of pancreatic insulin is influenced by blood glucose levels and is increased during hyperglycemic states, the remaining beta cells are subjected to a continuous chronic stimulation.

In spite of these diverse approaches to the study of experimental diabetes, the etiology and pathogenesis of glycogenic infiltration and possible deleterious effects of its presence have not been completely elucidated.

Duff and Toreson¹⁵ have described prevention and reversal of glycogenic accumulations in beta cells of alloxan diabetic rabbits by the administration of insulin. Since these changes occurred despite the concomitant presence of hyperglycemia they postulated that hypoinulinemia plays a more important role than hyperglycemia in the pathogenesis of glycogen infiltration. More recently Lazarus and Volk⁹ have suggested that glycogenic infiltration of beta cells merely represents one component of the generally increased glycogen deposition in diabetes and is a reflection of hyperglycemia rather than a degenerative change. In the present investigation glycogenic accumulations in beta cells have been studied in a variety of diabetic states, utilizing the greater resolution and magnification obtained with the electron microscope.

MATERIALS AND METHODS

A. Four spontaneously diabetic and several normal Chinese hamsters were obtained from Dr. George A. Yerganian.* Three of the diabetic as well as the normal animals were killed and their tissues processed as described below. A biopsy of the pancreas was obtained from the fourth diabetic hamster. At the time there was more than 2 per cent sugar in its urine. Subsequently this animal was treated with increasing amounts of NPH insulin (1 to 12 units daily) until the glucose content of the urine was reduced to less than 0.1 per cent. A second biopsy was taken nineteen days after the initiation of insulin administration and after 0.1 per cent or less of sugar was present in the urine for nine consecutive days.

B. Fifteen adult cats of both sexes were given intraperitoneal injections of glucose varying from 8.5 to 25 gm. of glucose/kg./day, three times daily for periods extending from thirty-six hours to fifteen days. The injection medium consisted of a 25 per cent solution of dextrose in normal saline which contained 125 mg. of thiamine HCl/liter to prevent the induction of a rela-

tive deficiency of the vitamin.

C. Growth hormone,* 4 to 6 mg. per day, was administered subcutaneously to partially depancreatized adult cats for a period of fourteen days. The material included in the present study was taken from one of these diabetic cats twenty-four hours following cessation of therapy.

D. Severe diabetes was produced in adult rabbits by the intravenous administration of 150 mg./kg. of a 5 per cent aqueous solution of alloxan. Blood sugar levels of 186 to 500 mg. per 100 ml. persisted for periods of one to three months until the animals were killed by an overdose of Nembutal.

In each of the above experiments, tissue for electron microscopy was fixed in 1 per cent osmic acid dichromate fixative buffered to pH 7.6²⁰ for one hour at room temperature. The tissue was then dehydrated through a series of graded ethanol solutions to absolute ethanol and embedded in methacrylate. Sections were cut with glass knives on a Servall Porter-Blum microtome after localization of islets in thick (2 μ) sections by phase microscopy. Thin sections were examined and photographed with RCA electron microscopes, models 2C and 2E. Electron micrographs were taken at original magnifications of 1,000 to 7,000 diameters and enlarged photographically as desired.

Blocks of pancreatic tissue were taken for light microscopy and fixed in either Bouin's solution or Zenker formol. Paraffin sections were cut and stained with hematoxylin and eosin (H & E) and aldehyde fuchsin.²¹ Glycogen was demonstrated by the periodic acid Schiff (PAS) method²² with control sections incubated in salivary amylase.

Determination of levels of blood sugar was done by the method of Somogyi.²³ Tes-Tape[†] was used to estimate glycosuria.

OBSERVATIONS

A. *Normal.* The normal ultrastructure of beta cells has been described in the rabbit and cat by Lacy^{24,26} and in the cat by Bencosme.²⁷ Beta cells in each of the three species studied possess numerous well-developed mitochondria, prominent lamellar ergastoplasm and ribonucleic acid (RNA) granules, and varying amounts of Golgi material. Their secretory granules, however, differed strikingly both numerically and morphologically.

Secretory granules in the beta cells of the rabbit

*Children's Cancer Research Foundation, Boston, Massachusetts.

*Gifts of Endocrine Study Section and Armour Pharmaceutical Company.

†Eli Lilly and Company.

were round to oval in shape and were located within sacs from which they were separated by narrow spaces (figure 1). They were relatively sparse in comparison to those of the cat and hamster. Peculiar to the rabbit were perinuclear accumulations of fine fibrillar material which extended irregularly throughout the cytoplasm of the beta cells. Beta granules in the hamster were round to oval in shape, moderately dense, and loosely enclosed within large membranous sacs. Two components were usually discernible in the beta granules of the cat. Typically a central elongated, dense, crystalloid structure was surrounded by less dense homogeneous material both of which were contained within a membranous sac (figure 2). Occasionally round or oval granules were seen, and more than one was present within a sac.

Alpha cells in all three species were similar in appearance and were characterized by their numerous round, dense secretory granules (figure 2). Mitochondria were fairly numerous, and lamellar ergastoplasm was present. Duct cells were also of similar appearance in all three species. Mitochondria were less numerous than in alpha or beta cells and both particulate and lamellar ergastoplasm were inconspicuous components of the cytoplasm.

B. Glycogen accumulation in experimental animals.

DIABETIC HAMSTERS

Light microscopy. Beta cells were partially degranulated and contained varying amounts of particulate PAS-positive material. In occasional beta cells nuclei were shrunken and large, clear cytoplasmic vacuoles

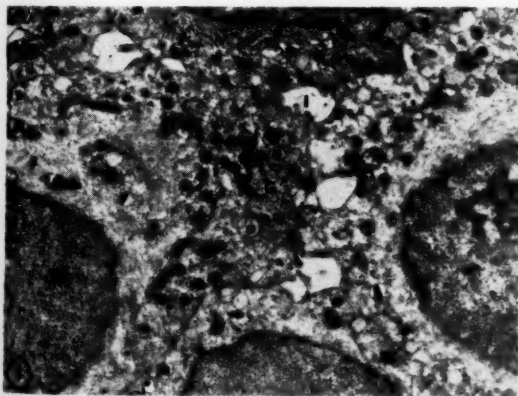


FIG. 1. Electron photomicrograph of portions of several normal rabbit beta cells. Note the perinuclear accumulations of fibrillar material (F). Nucleus (N), secretory granules (G), intercellular spaces (I). Approximately $\times 10,364$.

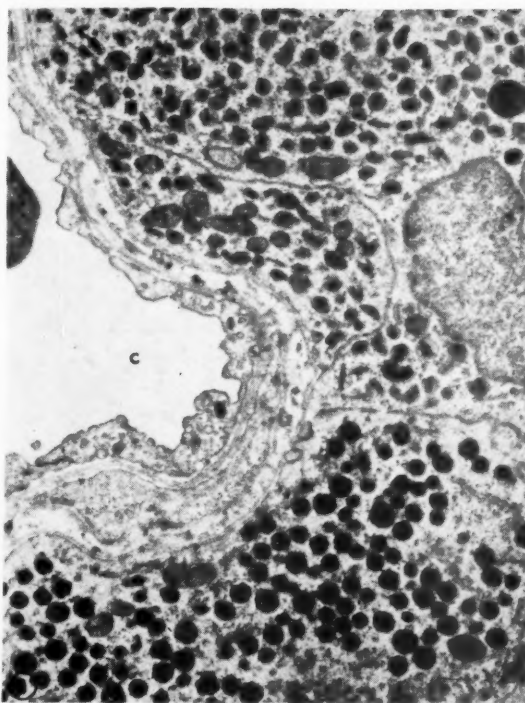


FIG. 2. Electron photomicrograph of portions of normal alpha (lower) and beta cells (upper) of the cat. Note the two components (central crystalloid structure surrounded by less dense homogeneous material) of the secretory granules of the beta cell in contrast to the round uniformly dense granules of the alpha cell. Capillary lumen (C). Approximately $\times 15,833$.

were present in addition to the PAS-positive material (figure 3). Alpha cells were normal in appearance and glycogen was not discernible in either alpha or duct cells.

Electron microscopy. Beta cell degranulation and focal as well as massive accumulations of glycogen were clearly evident by electron microscopy. The glycogen was present in the form of aggregates of moderately dense, branching granular material. These accumulations varied from small focal deposits which displaced organelles locally to masses which nearly filled the cytoplasm (figure 4). In the latter instances particularly, the ergastoplasm was moderately dilated. Vacuolation of mitochondria and marked dilatation of ergastoplasm were seen in other cells which contained little if any discernible glycogen (figure 4). Both glycogen infiltration and dilatation of ergastoplasm appeared to be associated with beta cell degranulation since neither was seen in its absence.

The large vacuoles devoid of glycogen which were

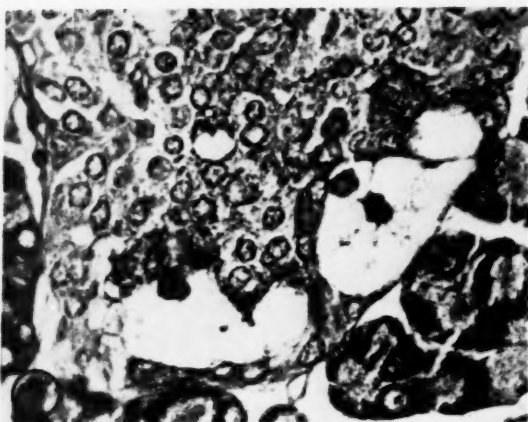


FIG. 3. Photomicrograph of glycogen and cytoplasmic vacuoles in beta cells of a diabetic Chinese hamster. PAS. Approximately $\times 452$.

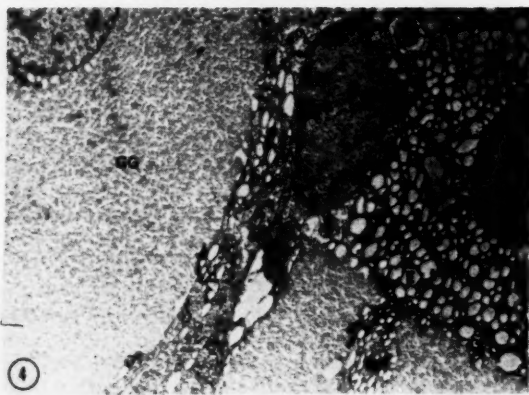


FIG. 4. Electron photomicrograph showing massive glycogenic accumulations (GG) in the beta cells on the left and marked dilatation of ergastoplasm (E) and vacuolation of mitochondria (M) in beta cell on the right. Diabetic Chinese hamster. Nucleus (N). Approximately $\times 4,030$.

seen by light microscopy were not evident in material examined with the electron microscope. This vacuolation was undoubtedly a consequence of autolysis and poor fixation of glycogen which was preserved, however, in tissues processed for electron microscopy, because fixation was initiated immediately at the time of death and the small size of the blocks facilitated rapid penetration of osmium tetroxide.

Effects of insulin administration. Nineteen days after the initiation of insulin therapy a biopsy of the pancreas was taken from one diabetic hamster. The content of glucose in the urine had been approximately 150 to 200 mg. daily prior to insulin and was reduced

to five to six mg. daily during therapy. Scarcely any glycogen was discernible in beta cells in the biopsy specimen, and granules were sparse. Mitochondria were much more numerous than in the previous biopsy. Numerous membranous sacs also were present and contained small amounts of homogeneous or slightly fibrillar material. In occasional cells, Golgi vesicles were dilated and appeared to be increased in number. Lamellar ergastoplasm and particulate RNA granules were also increased in amount.

HYPERGLYCEMIC CATS

Chronic hyperglycemia. Prolonged hyperglycemia (250 to 280 mg./100 ml.) was produced in cats by giving repeated intraperitoneal injections of glucose. By light microscopy varying amounts of glycogen (figure 5) were demonstrable in the islets of all injected animals including one animal which received only five injections within a period of thirty-six hours. Little, if any, glycogen was discernible in ductular epithelium. Slight to moderate degranulation of beta cells occurred in most animals and was inversely proportional to the degree of glycogen infiltration. The remaining granules appeared to be localized near the capillary margins of the cells.

By electron microscopy abnormal accumulations of glycogen were discernible only in beta cells. When present in small amounts it has the appearance of delicate branching strands of material scattered diffusely throughout the cytoplasm (figure 6). When larger amounts were present discrete accumulations were formed and cytoplasmic organelles were displaced (fig-

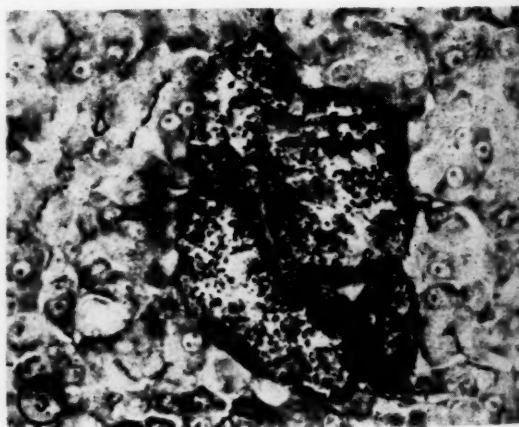


FIG. 5. Photomicrograph of glycogen in an islet from a cat which received 16 gm. glucose/kg./day for sixteen days. PAS. Approximately $\times 452$.

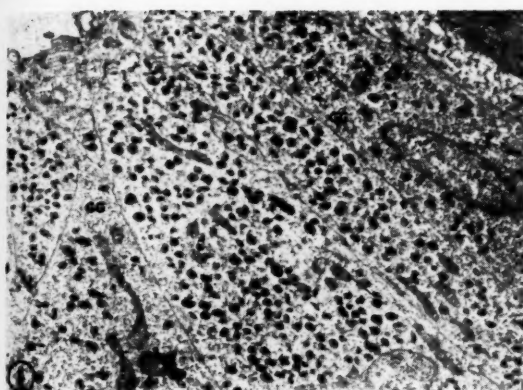


FIG. 6. Electron photomicrograph of portions of several beta cells from the same animal. Delicate branching strands of glycogen (GG) are most prominent in partially degranulated cells. Approximately $\times 10,364$.

ure 7). In some cells, the strands of glycogen appeared to be spread apart and separated by clear spaces. The degranulation of beta cells observed by light microscopy was confirmed and found to be most striking in cells containing relatively large amounts of glycogen. Cells containing no discernible glycogen possessed a normal complement of granules, a much higher proportion of which, however, lacked the central crystalloid component. Striking changes took place in the ergastoplasm of the beta cells of these animals. Particulate RNA granules were increased in number throughout the cytoplasm and concentric "fingerprint-like" whorls

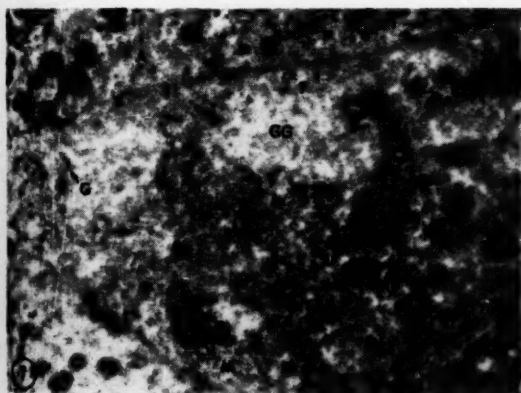


FIG. 7. Electron photomicrograph of an almost completely degranulated beta cell from a cat which received 14 gm. glucose/kg./day for eight days. Cytoplasmic organelles are displaced by accumulations of glycogen (GG). Beta granule (G), portion of alpha cell (A), plasma membrane (P), mitochondria (M). Approximately $\times 19,576$.

of lamellar ergastoplasm were observed in the basilar portions of some cells (figure 8).

Growth hormone. Preliminary observations on partially pancreatectomized cats made diabetic by administration of growth hormone revealed extensive accumulations of glycogen in the islets (figure 9). Although occurring primarily in the islets, it was also present in centro-acinar cells and ductular epithelium (figure 10). Sections stained by aldehyde fuchsin showed marked degranulation of beta cells.

Electron microscopy. Focal and diffuse accumulations of glycogen were observed in beta cells (figure 11),

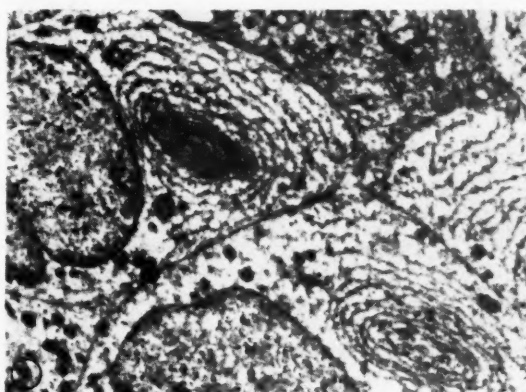


FIG. 8. Electron photomicrograph of portions of several beta cells from a cat which received 15.5 gm. glucose/kg./day for eight days. Numerous concentric lamellae of organized ergastoplasm (E) are present in the basilar portions of these cells. Nucleus (N). Approximately $\times 17,273$.

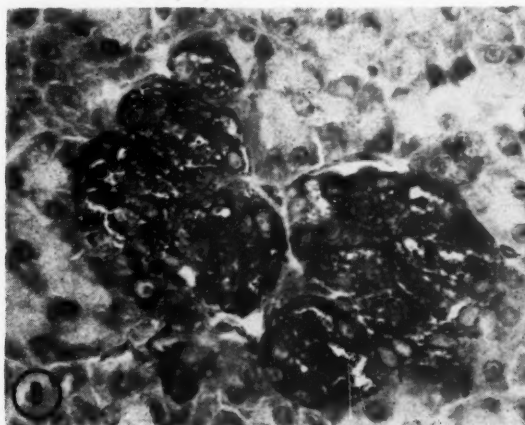


FIG. 9. Photomicrograph of glycogen in an islet from a cat which received 4 mg. growth hormone daily for fourteen days. PAS. Approximately $\times 452$.

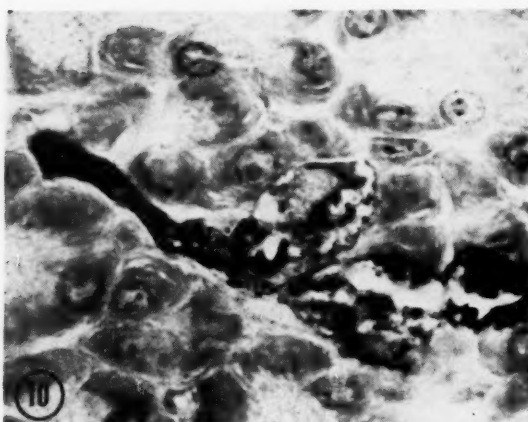


FIG. 10. Photomicrograph of glycogen in ductular epithelium of the same animal. PAS. Approximately $\times 905$.

whereas those occurring in ductular and centro-acinar cells were predominantly diffuse and poorly defined. Although present in much larger amounts, the morphology of the glycogenic deposits in these animals was identical to that seen in cats following chronic glucose administration. Beta cell degranulation was more pronounced and Golgi vesicles were dilated and more numerous than in cats receiving injections of glucose.

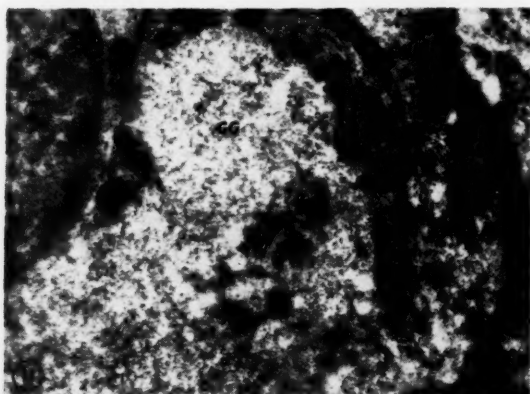


FIG. 11. Electron photomicrograph of a portion of a beta cell from the same animal. Note the delicate branching character of the glycogen (GG). Approximately $\times 25,909$.

ALLOXAN DIABETIC RABBITS

Light microscopy. Although the islets were decreased in number and quite small, they were easily located in sections stained by the PAS technic. The reaction of beta cells and ductular epithelium in these sections was

strongly positive for glycogen (figure 12). Very few granules were discernible in aldehyde fuchsin stained sections.

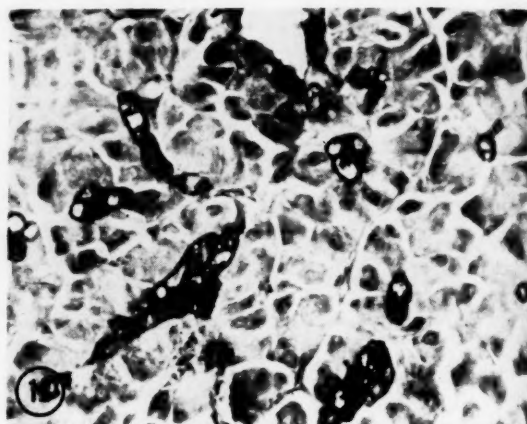


FIG. 12. Photomicrograph of glycogen in ductular epithelium and small islets in an alloxan diabetic rabbit. Approximately $\times 452$.

Electron microscopy. Beta granules varied considerably in density and many appeared to be slightly "smudged" (figure 13). Margination of granules next to the plasma membranes was striking in some cells (figure 13). Numerous empty or partially empty sacs containing amorphous material were present adjacent to the plasma membrane suggesting that the granules were undergo-

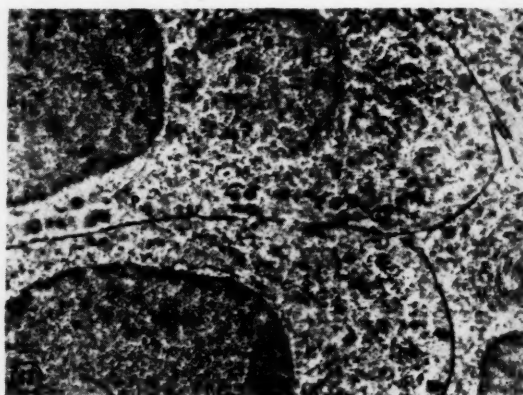


FIG. 13. Electron photomicrograph of portions of four beta cells from the same animal. The granules (G) vary considerably in density and are "marginated" adjacent to the plasma membrane (P). Partially empty sacs (S) contain amorphous material. Small foci of glycogen (GG) are scattered throughout the cytoplasm. Concentric whorls of lamellar ergastoplasm (E) are present in the beta cell in the right lower corner. Approximately $\times 8,636$.

ing dissolution. Whorls of lamellar ergastoplasm and an increase in particulate RNA granules were also prominent in these cells. Accumulations of fibrillar material appeared to be decreased in amount, but were discernible in most cells.

Accumulations of glycogen were very prominent in both beta cells and ductular epithelium (figures 14-17). In beta cells, accumulations of glycogen were inversely proportional to their content of secretory granules. The appearance of this glycogen differed slightly from that seen in the cat, but was similar to that described in the hamster. Granules of glycogen were larger and less dense than particulate RNA granules (figure 16). Numerous small islets composed solely of alpha cells were also seen by electron microscopy. They appeared to be normal and contained no discernible glycogen.

DISCUSSION

From these observations it is clear that the degree of glycogenic infiltration varies considerably from one beta cell to the next even within the same islet, and that glycogen deposition occurs only in those cells manifesting evidence of increased secretory activity.

Such evidence included varying degrees of degranulation, decreased density of granules suggestive of dissolution, and margination of remaining granules. An inverse relationship exists between the degree of granulation and the extent of glycogen deposition within individual beta cells. It is of interest that the deposition of glycogen occurs concomitantly with degranulation rather than as a consequence of it.

The increased prominence of both lamellar and par-

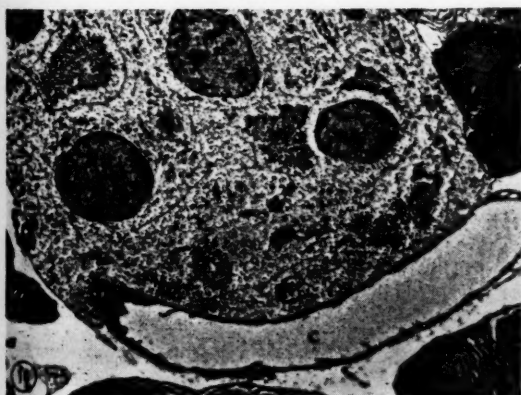


FIG. 14. Electron photomicrograph of a small islet in an alloxan diabetic rabbit. Numerous aggregates of glycogen (GG) are present in the cytoplasm of these cells. Nucleus (N), capillary lumen (C). Approximately $\times 2,879$.

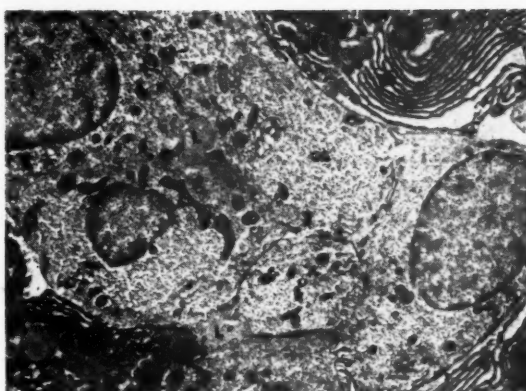


FIG. 15. Electron photomicrograph showing extensive accumulation of glycogen (GG) in ductular epithelium of alloxan diabetic rabbit. Lumen of duct (L), acinar cells (A). Approximately $\times 5,758$.

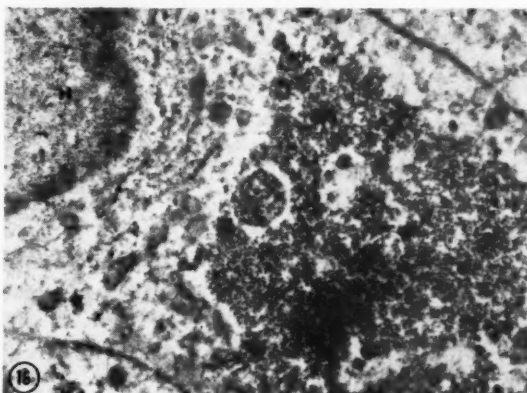


FIG. 16. Electron photomicrograph of glycogen in a beta cell in the same animal. Granules of glycogen (GG) are larger and less dense than RNA particles (\uparrow). Nucleus (N). Approximately $\times 23,030$.

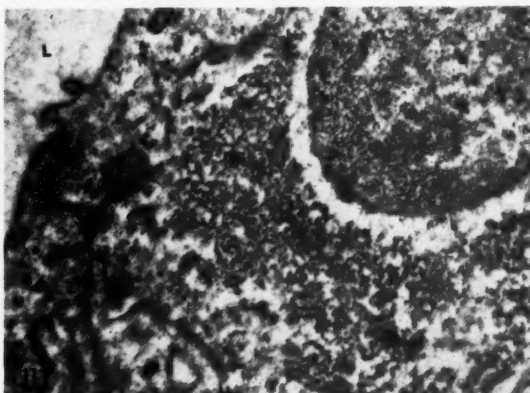


FIG. 17. Electron photomicrograph of glycogen (GG) in a duct cell of the same animal. Lumen of duct (L). Approximately $\times 23,030$.

ticulate ergastoplasm constitutes further evidence of increased secretory activity. The concentric whorls of ergastoplasm are reminiscent of those described by Weiss²⁸ in pancreatic acinar cells of mice which had been refed following starvation. They appeared to be associated with the formation of new zymogen granules. Palade and Siekevitz^{29,30} have done much to elucidate the relationship between RNA granules and protein synthesis. They have shown that incorporation of radioactive amino acids is much higher in tissues such as liver and pancreas, which contain numerous RNA granules and are actively involved in protein synthesis, than in tissue such as skin. The activity is first localized in the microsomal fractions (composed of fragments of lamellar and particulate RNA granules), and is later concentrated in the secretory product of acinar cells of the pancreas.

Thus two factors would appear to be of importance in regulating glycogenic infiltration in beta cells: (1) Hyperglycemia seems essential because pathological accumulations of glycogen are not known to occur in its absence, even though the beta cells are markedly stimulated. (2) Alterations in the metabolic state of the individual beta cell associated with increased secretory activity probably compel it to synthesize and store glycogen.

It should be emphasized that in the material observed definite changes indicative of degeneration, e.g., pyknosis and fragmentation of nuclei, dissolution of plasma membranes, ballooning of mitochondria, were not seen in association with accumulations of glycogen even when the latter were massive.

Since accumulations of glycogen develop in beta cells manifesting evidence of increased secretory activity, it is of interest to consider in what way alterations in protein synthesis might affect the carbohydrate metabolism of these cells.

Studies pertinent to this subject have been performed by Holme and his associates^{31,32} who have shown that an inverse relationship exists between glycogen synthesis and storage, and protein synthesis in *Escherichia coli*. When *E. coli* are grown in nitrogen-deficient media rich in carbon and energy sources, net protein synthesis is markedly reduced, whereas glycogen synthesis and storage are greatly increased (up to 20 per cent of the dry weight of the cell). The addition of a nitrogen source to the media results in an increased rate of synthesis of nitrogen-containing compounds and a decrease in the cellular content of glycogen. These effects indicated that the increased synthesis of glycogen was the consequence of reduced synthesis of protein because

of a lack of nitrogen precursors.

The environment in and around the beta cell may be quite analogous to that in the *in vitro* experiment described. Certainly the extracellular fluid is rich in a carbon and energy source (hyperglycemia). Increased secretory activity might well lead to relative depletion of insulin precursors and an imbalance between the rate of protein synthesis and carbohydrate uptake. If the enzymatic constitution of the beta cell is comparable to that of *Escherichia coli*, then one might well expect glycogen to be synthesized from the excess supplies of glucose.

Accumulations of glycogen in the rabbit and hamster were composed of aggregates of coarse granular material with some suggestion of "branching" (figure 16). In contrast glycogen in the cat was seen as accumulations of delicate branching amorphous material (figure 11). It is doubtful that these differences are due to variations in fixation, since all of the tissues were processed in the same manner. On the other hand, they may well represent differences in molecular weight and/or degrees of molecular asymmetry. Evidence that such differences do exist in liver glycogen in cats and rabbits has been reviewed by Manners³³; the molecular weight of glycogen in cat liver is approximately eight times that in the rabbit.

Thus far, nothing has been said concerning the mechanism of glycogen deposition in ductular epithelium. The appearance of glycogen within ducts is identical to that seen in beta cells in the same species, suggesting similar mechanisms of synthesis. But ductular cells do not, of course, contain any type of secretion granule. Glycogenic infiltration in beta cells can be correlated with alterations in functional activity whereas no definitive function has been elucidated for duct cells. Volk and Lazarus⁷ have emphasized that in experimental diabetes in the rabbit, glycogen infiltration in ductular cells usually precedes and is more marked than that occurring in beta cells. It would appear then that the etiology of glycogen deposition in ductular epithelium may be different from that in the beta cell. The presence of glycogen in duct cells during hyperglycemia, and its absence in alpha and acinar cells may simply be a reflection of cellular differences in enzymatic organization and metabolism of glucose.

Differences in the enzymatic activities of beta ductular and acinar cells have recently been demonstrated histochemically by Lazarus³⁴ and quantitatively by Lacy³⁵ using the microdissection technics of Lowry. Lazarus was able to demonstrate that glucose-6-phosphatase activity was much higher in beta than in ductular and

acinar cells. Lacy has found that lactic acid dehydrogenase activity in duct cells is approximately five times as great as in islets and ten times as great as in acinar cells.

Thus it would appear that glycogen accumulation in both beta cells and ductular epithelium is related to the enzymatic organization of these cells, and in the former to changes associated with increased secretory activity. It is hoped that further electron microscopic and micro-enzymatic studies of beta cells will elucidate the enzymatic interrelationships between carbohydrate metabolism and insulin synthesis, storage and release.

CONCLUSIONS AND SUMMARY

The appearance of abnormal accumulations of glycogen in beta cells and ductular epithelium has been described in a number of experimentally induced diabetic states.

The appearance of glycogen in the hamster and rabbit was identical and consisted of moderately dense aggregates of branching granular material. Glycogen in the cat had a slightly different appearance and was seen as delicate branching strands of amorphous material. Accumulations of glycogen in ductular epithelium were morphologically identical to those seen in beta cells of the same species in each case.

Glycogen deposition in beta cells occurred during degranulation rather than as a sequel to it, and appeared to be related to changes associated with increased secretory activity in the presence of hyperglycemia.

In the present study degenerative changes were not seen in association with accumulations of glycogen even when the latter were massive. Glycogen was not seen in either alpha or acinar cells, both of which were normal in appearance in all other aspects as well.

The pathogenesis of glycogen deposition in beta cells and ductular epithelium is discussed in relation to differences in functional activity and enzymatic constitution.

SUMMARY IN INTERLINGUA

Microscopia Electronica de Alterationes Glycogene del Cellulas Beta in Diabete Experimental

Es describe le apparition de accumulationes anormal de glycogeno in le cellulas beta e in le epithelio ductular in un numero de statos diabetic de induction experimental.

Le apparition de glycogeno in hamster e conilio esseva identic e consisteva de moderatemente dense aggregatos de brancate materia granular. In le catto le glycogeno habeva un apparentia levemente differente,

presentante se como delicate strias brancate de materia amorphe. Le accumulationes de glycogeno in le epithelio ductular esseva morphologicamente identic con le accumulationes vidite in le cellulas beta del mesme specie. Isto valeva sin exception.

Le deposition de glycogeno in cellulas beta occurreva durante le disgranulation plus tosto que como sequella de illo. Illo pareva esser relationate a alterationes associate con un augmento del activitate secretori in le presentia de hyperglycemia.

In le presente studio, alterationes degeneratori non esseva notate in association con accumulationes de glycogeno, mesmo quando istos esseva massive. Nulle glycogeno esseva trovate in cellulas alpha e nulle in cellulas acinar. Ambe istos esseva de apparentia normal etiam in omne altere respectos.

Le pathogenese del deposition de glycogeno in cellulas beta e epithelio ductular es discutate in relation a differentias del activitate functional e del constitution enzymatic.

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New Statistics on Diabetes

Diabetes is the subject of one of the latest in the series of statistical reports covering separate health-related topics which are prepared by the U. S. National Health Survey. This report, released just as this issue of *DIABETES* is going to press, is based upon information collected in the continuing nation-wide sample of households in the National Health Survey. The report on diabetes relates to the findings in the twenty-four month period, July 1957—June 1959, on about 235,000 persons from 73,000 households.

On the basis of the survey, the prevalence of known diabetes in the United States is estimated to be about 1,500,000 or nine per 1,000 population. This figure relates to those cases which have been diagnosed, about which the family has been informed, and which are believed by the family to have been present in the year

prior to the household interview. The figure, moreover, may represent some degree of understatement inasmuch as the survey did not include the institutionalized population (homes for the aged, nursing homes and other resident institutions).

Further details and an analysis of the report which cover both prevalence and disability from diabetes by age, sex, and status of medical care, will be presented in a forthcoming issue of *DIABETES*. Interested readers who want to review the findings of this important report may obtain a copy from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D.C. (price 25¢). The title of the publication is *Health Statistics from the U. S. National Health Survey: Diabetes reported in interviews, United States, July 1957—June 1959* (Series B—No. 21).

Blood Lipid and Protein Levels in Juvenile Diabetes Mellitus

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In recent years much emphasis has been placed upon the relationship of hyperlipemia to the development of atherosclerosis. The tendency for vascular disease to develop prematurely in patients with diabetes mellitus is well known. Therefore, it seemed pertinent to study the serum lipid and protein levels in patients with juvenile diabetes mellitus in whom there was no clinical evidence of arterial disease. Our findings are presented herewith and discussed in the light of other reports.

CLINICAL MATERIAL AND METHODS

Fifty patients with juvenile diabetes mellitus were studied in private practice and in the diabetes outpatient clinics and hospitals from which this paper originates. The severity of the diabetes, degree of control, and duration of the disease varied with each patient. There were twenty-four males and twenty-six females, ranging from nineteen months to sixteen years of age. The shortest duration of the disease was three weeks and the longest was eleven and one-half years. The patients were divided into two groups: those whose duration of diabetes was less than five years, and those who had diabetes longer than five years. There were fifteen males and sixteen females in the former group, and nine males and ten females in the latter group.

None of the patients was hospitalized specifically for this study. All patients received Lente or NPH insulin, alone or in combination with Regular Insulin. In a few instances, mixtures of the Lente insulins were administered. The daily insulin dose was given in one injection before breakfast. The patients received a weighed diet of approximately 3 to 4 gm. of protein per kilogram of body weight, with a protein to fat to carbohydrate ratio of approximately 1:1:2. Activity was unrestricted. These patients tested their urine three to five times a day. Estimation of clinical control was based on the patient's well-being, freedom from symptoms, and a normal gain in weight. If acetone appeared

in the urine, or glycosuria appeared in more than half of the daily urine specimens, or mild reactions occurred, diabetes was considered to be unsatisfactorily controlled. In such an instance, readjustment of either the insulin dose and/or diet was made. Details of the routine of management employed may be found elsewhere.¹ Our criteria are similar to Keiding's² classification of the degree of control as "good, fair or poor."

Each patient was studied with the following laboratory tests: complete blood count; bleeding, coagulation, clotting, and prothrombin times; twenty-four-hour urine for quantitative albumin and sugar; fasting blood glucose, cholesterol, cholesterol esters, phospholipid, triglyceride, total lipid, lipoprotein (electrophoresis), total protein and A/G ratio, protein (electrophoresis), cephalin flocculation, and vitamin A level. Blood samples were drawn in the morning before the patient received his daily dose of insulin or had his breakfast. This battery of tests was done once in all our diabetic children. The few instances of incomplete determinations were due to technical laboratory difficulties.

LABORATORY METHODS

Total cholesterol and cholesterol esters were determined by the method of Schoenheimer and Sperry,³ and triglycerides by the method of Van Handel and associates.⁴ Blood for phospholipids was ashed in triplicate by a modification of the method described by Gortner,⁵ and the phosphorus measured by the method of Fiske and Subbarow.⁶ The total lipids were calculated from the above determinations.

Serum proteins were separated by filter paper electrophoresis in duplicate by the method of Durrum⁷ with the following modifications: Samples containing ten lambda of protein were placed on eight strips of Whatman 3-mm. paper and run for sixteen hours at five milliamperes in a 0.075 ionic-strength veronal buffer of pH 8.6. The proteins were stained with bromphenol blue and evaluated in an Analytrol scanner. The values were compared with normal children's values as reported by Oberman and associates.⁸

Lipoproteins were separated by paper electrophoresis

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in duplicate in the same manner with the use of fifty lambda samples that were prestained with Sudan black B by the method described by Bermes and McDonald.⁹ The results were read in the Analytrol scanner, and the quantity of lipid present was measured by the area under the curve, the result being a measure of the relative contribution of each lipoprotein component to the total lipid.

All values were compared with those for normal children as determined by Lever¹⁰ and in our laboratory (table 1). Blood sugar was determined by the Nelson-Somogyi method.^{11,12}

RESULTS

Normal values for chemical constituents of blood are given in table 1.

The results obtained for forty-nine juvenile diabetic patients are presented in table 2. One patient of the original group of fifty was omitted from statistical evaluation because chemical studies were performed at the initial phase of her disease prior to treatment. Of significance in the group as a whole is the elevated beta

TABLE 1
Normal values for chemical constituents of blood

Glucose		60-80 mg. per 100 ml.	Mean
Cholesterol	Age	Range (mg. per 100 ml.)	(mg. per 100 ml.)
	Newborn (cord)	54-120	79.3
	1-30 days	90-160	122.4
	2 months	87-169	126.5
	3 "	106-184	138.4
	4 "	142-178	154.4
	5-12 "	125-198	159.1
	2-6 years	144-188	172.7
	6-13 "	150-250	190.2
	13-16 "	148-392	209.8
Cholesterol esters		120-220	146
Phospholipid		160-310	240
Triglyceride		0-400	180
Total lipid		570-820	700
Cholesterol/phospholipid 0.85-0.94			
Electrophoresis of proteins*			
		Per cent of total protein	
Albumin		63.37±5.7	
Alpha ₁ globulin		3.43±0.6	
Alpha ₂ "		8.84±1.3	
Beta "		7.34±0.8	
Gamma "		17.02±4.5	
Electrophoresis of lipoproteins*			
		Per cent of total lipoprotein	
0 fraction		42.4±7.8	
Alpha "		15.4±5.6	
Beta "		42.3±4.7	

*Normal values for lipoproteins and proteins, as determined by paper electrophoresis, expressed as mean and standard deviations.

globulin fraction and the depressed gamma globulin fraction. All other protein determinations were within normal limits.

The mean blood glucose level for the total group was elevated, being 215 mg. per 100 ml. The group with diabetes of less than five years' duration had a mean level of 215 mg. per 100 ml. Those with diabetes of longer than five years' duration had a mean level of 214 mg. per 100 ml.

Complete blood count; bleeding, clotting, coagulation and prothrombin time; and vitamin A level were within normal limits for the entire group.

The cephalin flocculation test was negative in all but one patient. This was an eight-year-old boy with hepatomegaly who had had diabetes for six years. Additional laboratory findings for this patient include a cephalin flocculation of 3+ and hyperlipemia involving all components. Attempts are being made to reverse his present condition by instituting a low fat diet with the addition of safflower oil.

In the group whose duration of diabetes was under five years there were seven instances of hyperlipemia. Six of these were associated with acidosis in patients who are classified as "poorly" controlled. The remaining patient was the previously mentioned new diabetic who was in severe acidosis with a marked hyperlipemia. This girl was omitted from the statistical analysis because of the gross abnormality of her biochemical determinations at this initial phase of her disease. In five of the seven patients the serum lipids reverted to normal after correction of the metabolic acidosis, but in two the hyperlipemia persisted. One was a two and one-half year old boy who had had diabetes for one and one-half years and was well controlled; he is currently receiving safflower oil. The other is a one and one-half year old patient who is poorly managed, in part because of inadequate parental care.

There were four instances of hyperlipemia in the group whose duration of diabetes was each over five years. One case was associated with acidosis, and the lipid values returned to normal after therapy. Another is the patient previously described with an elevated cephalin flocculation. The other two patients are sixteen-year-old girls. One has had diabetes for eight years and is cooperative and well controlled. The other has had diabetes for five years, is uncooperative and poorly controlled. The inclusion of these patients with hyperlipemia is reflected in the somewhat elevated mean total lipid values reported in table 2. Results of lipoprotein electrophoresis and all other lipid determinations were otherwise within normal limits.

Cholesterol/phospholipid ratios were within normal range for the total group as well as for the groups whose duration of diabetes was less than five years and more than five years.

Coefficient of correlation was determined for glucose and cholesterol, and for glucose and beta lipoprotein levels. There was no significant correlation for either.

Quantitative analyses of twenty-four-hour urine specimens for glucose were variable and negative for albumin in all fifty patients.

Means, standard deviations, standard error of the means, standard error of the differences, and tests of significance were calculated. There was no statistically significant difference between the two groups for any parameter measured.

DISCUSSION

The results of a large series of standard biochemical determinations in patients with juvenile diabetes mellitus of varying duration indicate that in almost all instances these values were within normal limits. The three exceptions to these findings were the elevation of blood glucose, the elevation of the beta globulin fraction, and the insignificant depression of the gamma globulin fraction. The few instances of hyperlipemia observed were, in the majority of instances, associated with acidosis. Following therapy, in most of these cases there was a return to normal lipid levels. Furthermore, contrary to the report of Wolff and Salt,¹⁰ no relationship between glucose levels and cholesterol or beta lipoprotein levels was demonstrated.

These findings are in agreement with those of Chaikoff and associates,¹³ who found normal values for cholesterol, cholesterol esters, phospholipid, fatty acids, and total lipid in pediatric patients with diabetes mellitus regardless of the duration of the disease or amount of insulin taken. The observations of Keiding,² Dine and Jackson,¹⁴ and Wolff and Salt,¹⁰ indicating that lipid concentrations are frequently elevated in young diabetic patients with poor control, are substantiated by the findings of the present paper. The publications of Engelberg,¹⁵ DeWind and associates,¹⁷ Adlersberg,¹⁸ and Ianaccone and Kornerup,¹⁹ in which elevated lipid values were reported to occur in presumably nonacidotic adult diabetic patients, are not supported by the present observations of juvenile diabetic patients. These differences may well represent progress of the disease process.

The elevated serum beta globulin and depressed gamma globulin in this study are findings which we are unable to relate directly to any postulated abnormality of lipid metabolism.

TABLE 2

Biochemical results of forty-nine juvenile diabetic patients

Blood constituents	No. determinations	Mean mg. per 100 ml.	Standard deviation
Glucose			
Total group	49	214.7	95.4
0-5 yr. duration	30	215.4	96.2
Over 5 yr. "	19	213.6	94
Cholesterol			
Total group	49	207.7	63.2
0-5 yr. duration	30	219.1	66.4
Over 5 yr. "	19	189.8	60.2
Cholesterol esters			
Total group	49	146.3	45.4
0-5 yr. duration	30	155.0	48.3
Over 5 yr. "	19	132.6	44.2
Phospholipid			
Total group	44	265.3	90.2
0-5 yr. duration	26	264.5	86.4
Over 5 yr. "	18	266.6	91.8
Triglycerides			
Total group	46	84.7	51.8
0-5 yr. duration	28	86.6	48.2
Over 5 yr. "	18	81.7	53.7
Total lipid			
Total group	43	828.2	268.2
0-5 yr. duration	26	832.9	262.8
Over 5 yr. "	17	820.2	256.4
Cholesterol/phospholipid			
Total group	48	0.81	0.05
0-5 yr. duration	30	0.85	0.04
Over 5 yr. "	18	0.76	0.04
PROTEINS			
Serum albumin			
Total group	49	59.2	8.6
0-5 yr. duration	30	59.4	8.2
Over 5 yr. "	19	59.0	8.8
Alpha ₁ globulin			
Total group	49	4.65	1.5
0-5 yr. duration	30	4.80	1.2
Over 5 yr. "	19	4.44	1.4
Alpha ₂ globulin			
Total group	49	9.93	3.8
0-5 yr. duration	30	9.67	3.2
Over 5 yr. "	19	10.34	3.8
Beta globulin			
Total group	49	11.76	3.9
0-5 yr. duration	30	11.73	3.5
Over 5 yr. "	19	11.81	2.8
Gamma globulin			
Total group	49	13.38	4.0
0-5 yr. duration	30	13.36	3.6
Over 5 yr. "	19	13.42	3.8
Relative per cent of total protein			
Lipoproteins			
0 lipoprotein			
Total group	46	47.6	7.6
0-5 yr. duration	27	46.2	6.0
Over 5 yr. "	19	49.7	7.2
Alpha lipoprotein			
Total group	46	15.2	4.6
0-5 yr. duration	27	15.6	4.4
Over 5 yr. "	19	15.1	5.0
Beta lipoprotein			
Total group	46	35.9	6.4
0-5 yr. duration	27	37.1	5.8
Over 5 yr. "	19	34.2	6.8
Relative per cent of lipoproteins			

The failure to substantiate the reported correlation between beta lipoprotein and blood glucose levels would tend to cast doubt on the validity of the hypothesis that hyper-beta-lipoproteinemia develops when insufficient carbohydrate is available for metabolic needs. Furthermore, the postulated relationship between beta lipoprotein levels of juvenile diabetic patients and the development of atheroma¹⁰ would appear to be based on an unsound premise.

While the tendency of diabetic patients to develop premature arterial disease leading to occlusive coronary disease, retinopathy, and nephropathy is well known, the present study of treated diabetic children fails to demonstrate any of the biochemical abnormalities that have been suspected as pathogenetic factors in diabetic vascular disease.

SUMMARY

Blood lipid and protein determinations in fifty patients with juvenile diabetes mellitus were recorded. There was no statistical difference between those children whose disease had been present less than five years and those who had diabetes longer than five years. The only abnormal findings were an elevated blood glucose, elevated serum beta globulin fraction, and a decreased serum gamma globulin fraction.

There was no correlation between glucose levels and cholesterol or beta lipoprotein levels.

The question of the relationship of biochemical findings to the pathogenesis of arterial disease in juvenile diabetic patients is discussed.

SUMMARIO IN INTERLINGUA

Nivellos Sanguinee de Lipido e Proteina in Diabete Mellite Juvenil

Le nivellos sanguinee de lipido e proteina esseva determinate in cinquanta patientes con diabete mellite juvenil. Nulle statisticamente significative differentia esseva trovate inter le juveniles con diabete de un duration de minus que cinque annos e illes con diabete de un duration de plus que cinque annos. Le sol constata-tiones anormal esseva augmentos del glucosa sanguinee e del fraction de globulina beta in le sero e un reduction del fraction de globulina gamma in le sero.

Nulle correlation esseva trovate inter le nivellos de glucosa e le nivellos de cholesterol o de lipoproteina beta. Es discutate le question del relation inter le constata-tiones biochimic e le pathogenese de morbo arterial in juvenil patientes diabetic.

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Special Article

Diabetes Mellitus in Animals

A Review

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INTRODUCTION

Spontaneous diabetes mellitus has been reported in a number of animal species, including cattle, horses, pigs, sheep, dogs and cats. Reports of spontaneous diabetes in rodents were nonexistent until the recent discovery of an hereditary type in inbred strains of Chinese hamsters. Estimates of the relative frequency of spontaneous diabetes in larger domesticated animals vary considerably, although the reported incidence for dogs, for example, ranges from 1:260 to 1:800, and that for cats, approximately 1:1,000 to 1:1,500. Personal observations of some 30,000 dogs and cats would indicate that the disease is much more frequent, roughly 1:200 for dogs and 1:800 for cats. The relative frequency of diabetes in cattle, horses, pigs and sheep is unknown, but judging from the literature, the condition seems to be infrequent.

The present paper is intended as a review of the literature concerning spontaneous diabetes mellitus of animals; it also describes the clinico-pathologic findings in each species and compares them with those of human and experimental drug-induced diabetes.

LITERATURE

For general information on diabetes mellitus in larger domesticated animals, reference is made to textbooks of veterinary pathology and physiology, including those by Smith and Jones,¹ Dukes,² Hutty et al.,³ and Nieberle and Cohrs.⁴ A complete summary of older literature on diabetes in various animals was presented by Hjärre.⁵

Much of the older and more recent reference material has been surveyed by Wilkinson,⁶ who collected some fifty or sixty reports of spontaneous diabetes mellitus in a variety of animal species. He presents information regarding the normal anatomy of the pancreatic islets, their relative numbers, sizes and distribution

in various animals. In listing the available literature on diabetes according to species, the number of reports gives some indication of the relative frequency in various kinds of animals.

Diabetes in the horse has been reported by Preller,⁷ Heiss,⁸ Aellig⁹ and Krüger.¹⁰ Fröhner,¹¹ writing on canine diabetes, makes reference to two more cases. Diabetes mellitus in cattle has been described more frequently. Christensen and Schambye¹² surveyed bovine diabetes and a single case is reported by Hildebrand.¹³ Sharma¹⁴ mentions a case in a buffalo cow. Biester¹⁵ described diabetes in a pig and Baker, Reid and Owen¹⁶ reported on diabetic coma of feed-lot sheep. The largest amount of literature is available for the dog. An excellent summary was given by Freudiger and Köhler¹⁷ who also reported on a case in great detail. Other cases were published by Schindelka,¹⁸ Fröhner,¹¹ Krippel,¹⁹ Millar,²⁰ Aellig,⁹ Coffin and Christensen,²¹ Mayr,²² Saaticioglu and Atosy,²³ and Waddington,²⁴ who also commented on therapy of canine diabetes and gave details on insulin dosages and diabetic management. The report by Schlottbauer and Miller²⁵ is significant because of its description of nine cases (eight dogs and one cat) and excellent illustrations of islet changes. The paper also contains all references available up to that time and, in addition to the ones previously listed, those of Milks,²⁶ Cushing,²⁷ Milks and Stephenson,²⁸ Malherbe,²⁹ Christensen,³⁰ McBride,³¹ Vine,³² Pollok and Bauman,³³ Bloom and Handelsman,³⁴ Mettan and Craig,³⁵ are worthy of mention. Ricketts et al.³⁶ in an account of eight cases of spontaneous diabetes mellitus observed hydropic degeneration, glycogen deposition and beta-cell loss. They also found glomerular lesions of the Kimmelstiel-Wilson type which are not produced experimentally by induction of hyperglycemia.

A most beautifully illustrated and detailed treatise on diabetes of both dogs and cats was published by Hjärre.⁵ Aside from this outstanding investigation, there are a few cases reported on diabetes in cats, specifically. These include those of Keep,³⁷ McEvoy,³⁸ Rubarth,^{39,40} and Holzworth and Coffin.⁴ Fröhner¹¹ cites a case in a monkey.

Despite the fact that there is an extensive literature

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on experimental surgical and drug-induced diabetes in laboratory rodents, a search of the literature has revealed no cases of spontaneous diabetes. However, recently Meier and Yerganian⁴² observed an hereditary spontaneous diabetes in the inbred Chinese hamster.

Although the entire veterinary literature contains probably fewer than one hundred references to spontaneous diabetes mellitus, and most of them described only single cases or a few cases each, it is not to be inferred that this condition is rare. Some rough figures on incidence have already been given; routine clinical examination of all sick animals and postmortem studies would probably reveal a much greater frequency. This is evidenced by personal observations in a large sampling of dogs and cats.

CLINICO-PATHOLOGIC FINDINGS

Horses

Unfortunately, complete histopathologic examinations of diabetic horses are rare. Vacuolar-hydronic degeneration of the islets of Langerhans has been observed. In one case, "the pancreas had undergone an extensive degenerative process of colloid variety and appeared to have been the seat of several minute abscesses." These changes were interpreted as a sequence of an "atypical infection in the form of influenza" (viral disease of horses). Although gross pathologic changes were lacking in some horses, furunculosis was reported associated with diabetes mellitus in one horse. In another instance, atrophy of the pancreatic parenchyma with increase of interstitial connective tissue was noted; the islets were shrunken and disrupted. There was hepatomegaly from fatty dystrophy and the kidneys were enlarged.

The clinical picture was as follows: The fur was dull and rough; polyuria, glucosuria and polydipsia were severe. The appetite was usually good; however, the animals became extremely emaciated and sluggish. Some developed unilateral or bilateral cataracts with total blindness and, in one horse, cough and nasal discharge were observed. In two horses, in addition to bilateral cataracts, deep corneal ulcerations occurred.

Cattle

Diabetes mellitus is most commonly secondary to amyloidosis of the islets. As primary sources of chronic infection, pulmonary tuberculosis is most often mentioned. Occasionally hydronic degeneration of beta cells was observed. Decrease in size and number of islets has also been found. In some instances, extensive island infiltration with lymphocytes occurred in addition to vacuolar degeneration. In a severe case of chronic diabetes, gangrene and suppuration of the hoofs was re-

ported. The same cow gave birth to two calves, both of which died shortly after birth. No histologic examination was performed.

Weight loss despite excess food intake was observed; polydipsia and polyuria, with high specific gravity of the urine from glycosuria were reported. Eventually, the severely emaciated animals completely lost their appetite, became weak and died from acidosis as a result of ketonemia.

Sheep

Although there are no reports on either primary or secondary islet disease associated with diabetes mellitus, diabetic coma has been observed in feed-lot sheep. This condition has caused considerable loss in feed lots where the rapid method of fattening is practiced. The symptoms include sudden onset of convulsions, jumping, staggering, twitching, champing and frothing. Sometimes uncontrollable propulsion occurs. Finally, a comatose condition is observed before death. Blood and urine examinations revealed hyperglycemia and glycosuria in the absence of any pathologic lesions in dead animals. Treatment with insulin results in complete recovery. As a preventive measure, the amount of carbohydrates supplied in the ration is reduced, which necessitates, however, an increase in the feeding period to one-fourth longer than that of the rapid method.

Pigs

An interesting case¹⁶ of diabetes in a pig was thought to be secondary to pancreatitis preceded by an acute necrotic enteritis. The islets were "degenerated and necrotic" and congestion and fatty degeneration of the liver occurred; glycosuria was noted for four weeks, and attained a maximum concentration of 6.6 per cent. The pig also suffered from eczema accompanied by severe pruritis. Sections of the skin showed diminished size of the sebaceous glands and hyperkeratosis.

Dogs

The disease occurs in all age groups; however, there is a marked peak after eight years of age. The majority of diabetic dogs are females, especially neutered females. The microscopic findings most frequently observed are hydronic degeneration of the islets (figure 1), hyaline and amyloid changes. It has also been demonstrated that in diabetes in the dog, as in human diabetes, the argentophilic alpha cells of the islets increase in number. The proportion of alpha to beta cells, which is normally about 1:4 may be changed as abnormally as 3:4.5. Diminution in the number and size of the islets, and even almost complete absence of islets may be observed. In older dogs, even nor-

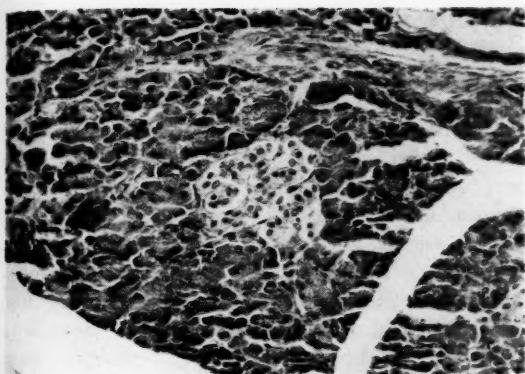


FIG. 1. Hydropic degeneration of an islet in a dog. Hematoxylin and eosin. $\times 154$.

mally, islets are relatively fewer than in young ones, indicating decreased ability of regeneration with aging. Islet sclerosis appears to follow changes which are either primarily degenerative or inflammatory. In dogs, diabetes is less frequently a disease of the islet tissue proper than a probable sequel to acute and chronic (relapsing) pancreatitis which is common in obese animals. In view of the fact that the canine pancreas usually has two excretory ducts, the main duct entering into the duodenum about 1 cm. posterior to the bile duct, pancreatitis is not thought to result from bile reflux. Rather, it has been suggested that since the pancreatic acinar tissue is actively involved in protein synthesis, a relative lack of lipotropic materials in obese animals may cause a metabolic disturbance and collapse of the pancreatic acini. The islets may become secondarily affected, following autodigestion and inflammation. Some dogs appear to be predisposed to repeated bouts of pancreatitis, diabetes occurring only during recurrent attacks. Histologically, various stages of acute and chronic inflammation are found. Symptoms of acute pancreatitis are abdominal pain and peripheral circulatory collapse. If a case persists for several days, there will be hyperglycemia due to destruction of island tissue. In chronic pancreatitis, polydipsia and polyuria frequently are first noted, or signs of digestive disturbances consisting of ravenous appetite or anorexia, prevail. In a few weeks' time, following apparent recovery, symptoms referable to diabetes mellitus with steatorrhea occur. Although the signs of pancreatic fibrosis are usually similar to those of chronic pancreatitis, the condition is of more gradual onset; the symptomatology is indistinguishable from cases of diabetes with primary islet cell lesions. It should be mentioned that in all cases of pancreatic carcinoma reported in the literature,

and from personal observation, diabetes has never been found in contrast to the situation in man.

The liver reveals, in all instances of diabetes, massive fatty dystrophy, the lipids being mostly anisotropic. Kupffer's cells are usually free of fat deposition. In the kidney, infiltration by glycogen appears frequently in the distal loop of Henle. Nephrosis, distal tubular dilation with hyalin (albumin) and cellular casts and glomerulosclerosis, are frequently observed. In the cardiovascular system, depending upon the duration of the diabetes, atherosclerosis and intimal proliferation may occur with obliteration of the lumen in some of the smaller arteries. Renal arteriosclerosis is a common finding. Occasionally, fatty infiltration into the parathyroid glands, skeletal musculature and reticulo-endothelium of the spleen may be seen. The endocrine glands, other than the parathyroids, are normal. Gastric ulceration may occur as a result of acidosis (ketonemia) and uremia which usually terminate a dog's life.

Cats

Diabetes is considerably more common in males than in females. Most of the observed cases revealed islet cell hyalinization and amyloidosis (figure 2). Islet disease secondary to pancreatic fibrosis has also been noted. Hydropic degeneration of beta cells also occurs. The fact that amyloidosis is definitely the most frequent alteration, especially in male cats, must be stressed. Amyloidosis is of a secondary type consequent to chronic inflammatory lesions elsewhere in the body. Since male cats, especially fighting tomcats, are subject to infectious foci such as bite wounds, alveolar abscesses of teeth, and tonsillitis, secondary amyloidosis and diabetes may be readily explained.

Chinese Hamster (*Cricetus griseus*)

Perhaps of greatest importance with respect to future research is the observation of spontaneous diabetes in Chinese hamsters maintained at The Children's Cancer Research Foundation in Boston. The evidence indicates that it is hereditary. Only certain of the inbred lines are affected and some reveal a higher incidence and greater severity of the disease than others. The mode of inheritance is currently being investigated in an intensive breeding program. It is obvious that there is tremendous value in having at hand an experimental animal in which genetic studies on diabetes can readily be made. Such a tool is also of importance in the screening and study of potentially hypoglycemic agents. Metabolic studies and insulin assays are now in progress; preliminary findings disclose insulin deficiency. Apart from the genetic aspects, the similarity of the pathologic findings to certain human changes and to

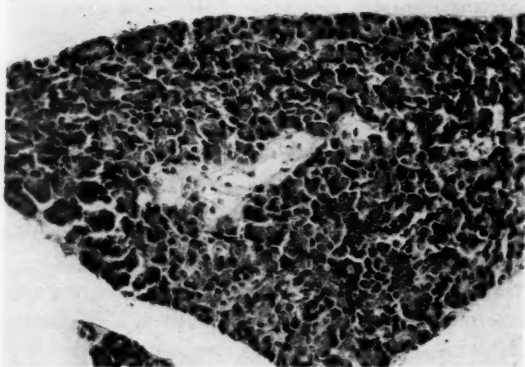


FIG. 2. Secondary amyloidosis and hyalinization of an islet in a male cat. Hematoxylin and eosin. $\times 154$.

alloxan diabetes in experimental animals is of interest.

In forthcoming papers the history of the origin of diabetes mellitus in Chinese hamsters will be presented. Also, the pathologic findings in offspring from diabetic parents based on sequential comparisons, with increasing age, of islet numbers, sizes and A:B ratios are being described. Interesting differences exist between diabetic, prediabetic and normal progeny. In one line of hamsters hydronephrosis is found to be an additional genetically determined expression. In another paper the electrophoretic serum changes and the use of elevated alpha-2 globulins as a genetic marker will be described. Finally the efforts to maintain a diabetic hamster colony aided by hypoglycemic therapy are being reported (papers by Meier, Yerganian and Green, in preparation).

COMMENTS

Diabetes mellitus in animals may be due either to primary (idiopathic) or secondary islet disease. Among the primary islet changes, "hydropic" degeneration and hyalinization prevail in Chinese hamsters, dogs, and also some cats of both sexes. Islet changes consequent to secondary amyloidosis are most frequent in cattle and male cats and acute and chronic pancreatitis commonly occur in obese dogs.

In man, particular significance attaches to the problem of etiology; three causes are generally listed—heredity, obesity, and hormonal-metabolic disturbances. Pancreatitis is less often a cause of diabetes. Although the importance of hereditary factors in the development of diabetes in man is stressed, genetic studies are limited. Considerable evidence has accumulated to indicate that the disease is of an hereditary nature in the Chinese hamster, probably transmitted by a recessive

trait. With respect to obesity and hormonal abnormalities as causes of diabetes in man, the analogy to similar observations in both dogs and fat feed-lot sheep is noteworthy.

Diabetes in animals, whether primary or secondary, is difficult to assess clinically. One or the other type may be suspected from previous experience. In this respect, the similarity of the pathologic findings in the Chinese hamster to those of man and experimental diabetes is of particular interest. The main cause of diabetes in the Chinese hamster is a disease of beta cells, and consists of degranulation, vacuolar ("hydropic") degeneration and beta cell deficiency. Implications have already been made as to the importance and significance of studies on diabetes in this laboratory animal to elucidate sequential morphologic changes and the pattern of inheritance. Although diabetic glomerulosclerosis occurs in animals, typical hyaline bodies or nodular intercapillary hyalinization is not observed. The lesions appear to be a more diffuse thickening of the capillary walls, rather than localized deposits. However, in the dog, glomerulosclerosis perhaps develops as further progression of renal arteriolar sclerosis which is quite frequently observed, associated with diabetes. Other than in the dog, renal arteriosclerosis is uncommon.

Various degrees of lipemia occur in all animals, except in the Chinese hamster; acetonuria is present in dogs and cats, but is severe in the hamster.

An attempt to focus attention on the matter of spontaneous diabetes in various animal species seems justified because of the relative lack of extensive investigations. It is hoped that this review will act as an incentive for further comparative pathologic studies, and more accurate objective statistical analysis.

SUMMARY IN INTERLINGUA

Diabete Mellite in Animales: Un Revista

Le occurrentia de spontanee diabete mellite se trova reportate in le litteratura pro un numero de species animal, incluse bestial, cavallos, porcos, oves, canes, e catts. Reportos de spontanee diabete mellite in rodentes esseva inexistente usque al recente discoperta de un typo hereditari in racias de derivation consanguinee de hamsters chine. Le estimationes del frequentia relative de diabete spontanee in le plus grande animales domestic varia considerabilemente. Pro canes, per exemplo, le reportate cifras de incidentia es inter 1:260 e 1:800; pro catts, inter 1:1.000 e 1:1.500. Observaciones personal del autor in un material de circa 30.000 canes e catts indicarea que le morbo es multo plus frequente, i.e. grossiermente 1:200 in canes e 1:800 in catts. Le

relative frequentia de diabete in bestial, cavallos, porcos, e oves non es cognoscite, sed—a judicare per le litteratura—le condition pare esser paucio frequente.

Le presente articulo es un revista del litteratura concernente spontanee diabete mellite in animales. Es etiam discutate le constataciones clinico-pathologic in le varie species supra-mentionate, e comparationes es facite con le constataciones in diabete human e pharmacogenic experimental.

Le objectivo del studio es stimular investigationes additional de pathologia comparative e analyses statistic plus accurate e objective.

ACKNOWLEDGMENT

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Effects of Insulin on the Complications of Diabetes and Pregnancy in the Rat

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The administration of insulin to pregnant diabetic women has largely solved the problem of fertility and maternal mortality.¹⁻⁴ On the other hand, the fetal mortality has remained higher than that in nondiabetic women.^{5,6}

Previous work from this laboratory has indicated that in untreated diabetic rats the growth of the fetuses is retarded.^{7,8} The length of the gestation period is significantly prolonged. If the prolongation is sufficiently great, the diabetic rats may show increased birth weights at the time of spontaneous delivery.

In the present study, insulin was administered to pregnant diabetic rats in order to determine whether it would correct these abnormalities.

METHODS

The animals used in this study were a subline of the Sprague-Dawley strain (Holtzman). They were virgin females whose age varied between 120 and 150 days and whose weight ranged between 230 and 280 gm. Alloxan was administered intravenously in doses of 40 mg./kg. body weight, in accordance with the method previously described (Lazarow and Palay⁹). All the diabetic rats were placed in metabolism cages, and fed a Purina fox chow diet. Food was supplied ad libitum; water was limited to 160 ml. per day. Diabetic animals were injected with 5 or 10 units of Protamine Zinc Insulin per kilogram of body weight per day, beginning on the third day after alloxan injection and continuing throughout the period of the experiment. No attempt was made to control the diabetes; insulin was administered primarily to increase fertility and the likelihood of pregnancy's going to term.

Blood samples were drawn in the morning at least twice each week and the blood sugar levels were determined by the Folin-Malmros micro blood sugar method.¹⁰ The twenty-four-hour urinary glucose excretion

was measured twice each week. The urine sugar was determined by the caramelization method of Somogyi,¹¹ and the twenty-four-hour glucose excretion was calculated.

A vaginal smear was made daily in each of the diabetic females and those animals showing estrus were placed with males of the same strain and watched until copulation took place. The time was recorded, and the mated females were caged separately.

The length of gestation between the time of witnessed mating and the time of witnessed delivery of the first young was measured. Beginning at twenty-one days and fifteen hours postcoitum, i.e., one hour prior to the earliest time of delivery in normal pregnancies (Wells¹²), the rats were watched at fifteen- to sixty-minute intervals. Fetuses or newborns were weighed individually. The newborns were weighed promptly after parturition and before they had nursed. The chainomatic balance was read to the closest milligram.

RESULTS

The sixteen treated diabetic rats were subdivided into three groups according to the amount of sugar excreted during twenty-four hours (table 1). It should be noted that the doses of insulin, arbitrarily selected, were suboptimal; they did not completely control the symptoms of diabetes. Four of sixteen animals excreted 0 to 1 gm. of glucose per day; seven of sixteen, 1 to 2 gm.; five of sixteen, 2 to 5 gm. The average blood sugar levels in these groups were 162, 254, and 343 mg. per 100 ml., respectively. Occasional rats showed hypoglycemia on a particular day, in which case the insulin dose was omitted that day.

In the sixteen treated diabetic rats, both the average length of the gestation period and the average birth weights were not significantly different from those in the normal group (table 1).

In those animals which showed a glycosuria of 2 gm. per day or less (Grades 1 and 2), there appeared to be a positive correlation between the birth weight and the insulin dose administered during the last one third of pregnancy (figure 1). Figure 2 shows that

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TABLE 1
The severity of diabetes versus birth weight, gestation age and litter size

Characterization of the diabetes	Number	Mothers			Number	Newborns		
		Average urine sugar (gm./day)	Average blood sugar (mg. per 100 ml.)	Average daily dose of insulin (units/kg.)		Average birth weight (gm.)	Average gestation age (hr.)	Average litter size
Grade 1	4	0-1	162	8.35	27	6.102	538	6
Grade 2	7	1-2	254	8.73	63	6.055	545	9
Grade 3	5	2-5	343	9.40	51	6.290	539	10
Normal rats	39		109*	0	289	6.224	542	10

*Observations on ten rats.

seven out of the eight treated diabetic rats which had one to four days of hypoglycemia (blood sugar less than 70 mg. per 100 ml.) had subnormal birth weights. The one of the eight with a birth weight above normal was a litter consisting of a single newborn (rightmost bar); this may have contributed to the overweight.

In analyzing the data on birth weight, it was noted that the variance in the treated diabetic series was much larger than that in the normal series. Overweight newborns were observed 3.6 times more frequently in the treated diabetic group than in the normal group (3.6 per cent versus 1 per cent, see table 2). On the other hand, underweight newborns were found forty-one times more often in the treated diabetic group than in the normal (12.3 per cent versus 0.3 per cent). All seventeen underweight newborns in the treated

DOSE OF INSULIN GIVEN TO MOTHER
VERSUS BIRTH WEIGHT OF OFFSPRING

Average urine sugar excretion

- ▲ Rat excreted 0-1 gm. per day
- Rat excreted 1-2 gms. per day
- Rat excreted 2-5 gms. per day

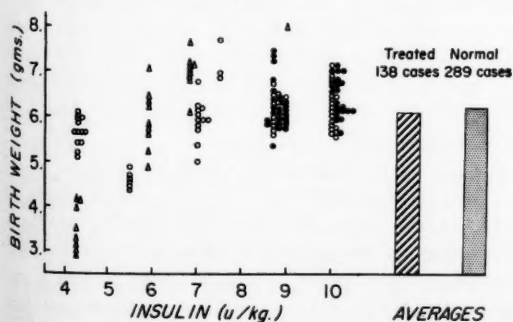


FIG. 1. The abscissa represents the average of the daily dose of Protamine Zinc Insulin administered during the last one third of pregnancy. The two bars show the average birth weights of all newborns of the two groups, these averages having been calculated from the mean weights of the litters.

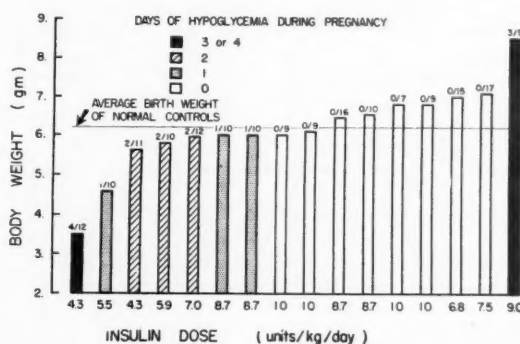
EFFECTS OF MATERNAL HYPOGLYCEMIA
ON BODY WEIGHT OF NEWBORNS

FIG. 2. The numbers above each bar compare the number of days hypoglycemia was observed with the number of times the blood sugar levels were determined during pregnancy. The abscissa represents the average of the daily dose of Protamine Zinc Insulin administered during the last one third of pregnancy.

TABLE 2

Overweight and underweight newborns in normal and diabetic groups*

Groups	Total number of newborns	Overweight, + 2 S.D. (7.384 gm. or more)		Underweight, - 2 S.D. (5.164 gm. or less)	
		Number	Per cent	Number	Per cent
Normal	289	3	1.0	1	0.3
Diabetic, O-term; insulin, O-term	138	5	3.6	17	12.3

*Overweight newborns are those whose weights exceed + 2 standard deviations from the mean for normal controls; underweight newborns are those whose weights are smaller than - 2 standard deviations from this mean.

diabetic group were from rats which had one or more episodes of hypoglycemia during the pregnancies.

In the experimental rats in which the diabetes was most nearly controlled by the insulin (Grade 1), hypoglycemia occurred frequently (table 3).

The average fetal mortality for the entire diabetic group was 2.5 times greater than that for the normal group (figure 3); the P value for this difference was less than .001. It was greater in rats with diabetes of Grades 1 and 2 than that in those of Grade 3. These

TABLE 3

Maternal blood sugar versus stillborns in sixteen pregnant rats

Diabetes	Individual rats	Blood sugar 70 mg. per 100 ml.		Stillborns
		mg. per 100 ml.	or less*	
Grade 1	1	149	4 of 12	5 of 7
	2	152	3 of 9	0 of 1
	3	162	2 of 10	2 of 9
	4	186	0 of 15	0 of 10
(19.6%)				
Grade 2	5	197	1 of 10	4 of 6
	6	252	2 of 12	2 of 12
	7	256	0 of 7	0 of 5
	8	259	2 of 11	3 of 13
	9	265	0 of 16	3 of 11
	10	266	0 of 9	0 of 13
Grade 3	11	281	0 of 17	1 of 3
	12	303	1 of 10	1 of 9
	13	316	0 of 9	0 of 10
	14	346	1 of 10	0 of 10
	15	368	0 of 10	0 of 11
	16	384	0 of 9	0 of 11
(4.2%)				

*Number of determinations during the entire pregnancy. Figures in parentheses indicate frequency of episodes of hypoglycemia.

animals of Grades 1 and 2 likewise showed more frequent episodes of hypoglycemia than those of Grade 3 (table 3).

Surprisingly, however, the fetal mortality was highest in the group of treated rats of Grade 1, i.e., those rats which had blood sugars ranging from 149 to 186 mg. per 100 ml. and which excreted 0 to 1 gm. of urine sugar per twenty-four hours. Also, the fetal mortality was lowest in the group of treated rats of Grade 3, i.e., those rats which had blood sugars ranging from 303 to 384 mg. per 100 ml. and which excreted 2 to 5 gm. of urine sugar per twenty-four hours.

DISCUSSION

The fetal mortality of 14.9 per cent in the insulin treated diabetic group was not as high as that found in two previous groups of diabetic rats which were reported by Kim, Runge, Wells and Lazarow.⁷ In the

THE SEVERITY OF DIABETES VERSUS STILLBIRTHS

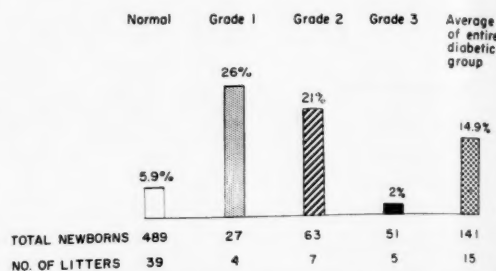


FIG. 3. The height of the bars represents the per cent of stillbirths in the normal and insulin treated diabetic groups. The left and right bars represent averages for the two groups. The three center bars represent subgroups of the treated diabetic group.

first of these two groups, the alloxan diabetes was induced on the twelfth day of pregnancy; the fetal mortality was 22.6 per cent. In the second group, the alloxan diabetes was induced before conception and the insulin treatment was given daily from day 0 to day 12 of pregnancy; the fetal mortality was 47.6 per cent. These observations suggest that in our present insulin treated diabetic rats the insulin reduced the fetal mortality.

Although the average gestation period and the average birth weight in the treated diabetic group were essentially normal, the average fetal mortality was nevertheless 2.5 times greater than that in the control group. The fetal mortality was greatest in those members of the insulin treated diabetic group which had an average blood sugar of 162 mg. per 100 ml., which excreted 1 gm. or less of urine sugar per twenty-four hours, and which had the greatest frequency of episodes of hypoglycemia (Grade 1). These episodes of hypoglycemia were consequences of the insulin treatment, and they might be a factor which contributed to the increased fetal mortality and to the seven subnormal birth weights which are shown in figure 2 (bars 1 to 7, reading from left to right).

SUMMARY AND CONCLUSIONS

Rats were rendered diabetic by alloxan injection prior to conception and treated with insulin throughout pregnancy. Although insulin treatment decreased fetal mortality, these treated rats nevertheless showed a significantly greater fetal mortality than the normal controls.

The treated diabetic animals with the greatest fetal mortality showed the least hyperglycemia and glycosuria. It is suggested that occasional hypoglycemia, occurring secondary to insulin administration, may be a factor in causing an increased fetal mortality and a

decreased fetal weight. The insulin treated group likewise showed increased frequencies both of overweight and of underweight newborns.

SUMMARIO IN INTERLINGUA

Le Effectos de Insulina Super le Complicationes de Diabete e de Pregnantia in le Ratto

Rattos esseva rendite diabetic per injectiones de alloxano ante le pregnantia e allora tractate con insulina usque al parturition. Ben que le tractamento con insulina reduceva le mortalitate fetal, iste tractate rattos monstrava nonobstante un significativamente plus alte mortalitate que normal animales de controlo.

Le tractate rattos diabetic con le plus grande mortalitate fetal manifestava le plus basse hyperglycemia e glycosuria. Es speculate que le hypoglycemia que occurre sporadicamente secundari al administration de insulina es possibilmente un factor in le causation del augmento del mortalitate fetal e del reduction del peso fetal.

Le gruppo tractate a insulina manifestava etiam augmentate frequentias de tanto neonatos a peso excessive como de neonatos a peso infranormal.

ACKNOWLEDGMENT

This study was aided by grants from the Medical Research Fund of the Graduate School and by Grants A-1244, A-1659 and A-1887 from the National Institute of Arthritis and Metabolic Disease, United States Public Health Service.

An alteration in the physical state of cortisol in the blood during pregnancy has been described by W. R. Slaunwhite and A. A. Sandberg (*J. Clin. Investigation* 38:384, 1959). Under certain conditions, plasma from pregnant women was shown by dialysis equilibrium to bind significantly more cortisol than plasma from non-pregnant women. The authors suggest that there is in pregnancy an increase in the plasma protein which normally is responsible for the binding of nearly all the cortisol of plasma.

The metabolism of aldosterone in pregnancy has attracted investigative interest because of the possibility that increased secretion of this hormone might be responsible in part for the salt retention which occurs in toxemia. Although the excretion of aldosterone in pregnancy has been measured by a number of methods, the most definitive study appears to be that of K. M. Jones, et al. (*Acta Endocrinologica* 30:321, 1959).

Tritiated aldosterone was injected into six pregnant women and a similar number of women who were not pregnant to permit an estimation of the secretion

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rate of aldosterone by measuring the specific activities of aldosterone and its metabolites in the urine. The mean total secretion rate for aldosterone in the normal woman so measured was 192 μ g. per twenty-four hours. Most of the pregnant women were found to be producing larger amounts of aldosterone; the mean adrenal secretory rate was estimated to be 582 μ g. per twenty-four hours. In the urine of normal women there was only about 0.44 μ g. of unconjugated aldosterone; a modest increase to 1.7 μ g. was present in the urine from pregnant women. In contrast, there was an eight- to ninefold increase in the aldosterone which is excreted in the urine as a labile conjugate which is hydrolyzed at room temperature at pH 1. This relative increase in the labile conjugate does not occur in the hyperaldosteronism which follows sodium restriction and may indicate an increase in this pathway of aldosterone inactivation.

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The Insurability of Persons with Diabetes Mellitus

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HISTORICAL

Twenty-one years ago no one known to have diabetes mellitus could procure life insurance in the United States or Canada. While one can point to occasional exceptions, that generalization essentially reflects the underwriting practice of practically all substantial life insurance companies on this continent at that time. Today the diabetic patient under good medical control, with no complicating condition, can procure life insurance in almost any of these companies. This shift in underwriting attitude stems primarily from the dramatic increase in the diabetic patient's life expectancy since the discovery of insulin in 1922. There has also been an extension of classes of impaired risks accepted for substandard life insurance, as clinical and actuarial studies of the long-term prognosis of medically impaired lives have progressed.

Although the Sun Life Assurance Company of England was insuring diabetic persons in 1924, the Manufacturers Life Insurance Company of Canada is generally credited with being the first on this continent actively to solicit and insure diabetic individuals, beginning this practice in 1940. During the next ten years this company had insured 550 diabetic persons, 76 per cent of them in the United States, 20 per cent in Canada and 4 per cent elsewhere.¹ By the mid-forties, a few other companies had begun to accept diabetic risks.

Shortly after 1950, the practice of issuing life insurance to persons with diabetes had become almost universal among life insurance companies in the United States and Canada. The experience of the Fidelity Mutual Life Insurance Company exemplifies the general trend. This company first offered insurance to diabetic persons in 1950. Today diabetes ranks fifth in frequency among medical impairments compatible with insurability on a modified premium basis and fourth among those which are declined insurance on any basis. It accounts for 5 per cent of cases limited to substandard insurance and 6 per cent of those declined.

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With diabetes, as with many other conditions, it is not the presence or absence of the impairment but its severity, complications and amenability to medical control that determine insurability. Furthermore, in the individual action, a number of factors beyond the main impairment may influence final decision.

EARLY REQUIREMENTS FOR INSURABILITY

In the beginning, no diabetic person was considered insurable unless he was between thirty and sixty years of age, had been under careful medical supervision for three years, was considered to be a diligent patient of good economic status, had remained under good medical control with urine sugar-free most of the time, and was found completely free from any evidence of vascular complication or associated impairment of health. Applicants with blood pressure above 140/90 were excluded. Albuminuria, cylindruria or eye ground change was considered cause for declination. Electrocardiogram and X ray of the chest were required for diabetic applicants over age forty, for those applying for \$10,000 of insurance, the maximum issued at first, and for any whose diabetes had existed ten years. Abnormality in electrocardiogram or X ray was ground for declination. As late as 1949, 59 per cent of diabetic applicants failed to meet these rather rigid requirements and were declined for insurance. Only 19 per cent were found acceptable. Many, 22 per cent, dropped their applications without completing all appraisal requirements.

PRESENT INSURABILITY REQUIREMENTS

Insurance appraisal of the diabetic applicant is initiated by preliminary information from the applicant himself, submitted by way of the company's agent. A résumé of patient's record is requested from the attending physician and constitutes a major source of clinical data essential to risk evaluation. The customary examination by a qualified company medical examiner is then arranged, unless preliminary review has definitely excluded possibility of risk acceptance.

To be insurable at the most favorable premium rates our company now offers to persons with diabetes, the following requirements must be met:

- Age fifteen to sixty-five
- Good economic grade
- Personal history and habits satisfactory
- Average or near average weight
- Blood pressure not over 140 systolic or 90 diastolic
- Diabetes duration not over twelve years
- Diabetes controlled by 60 units or less of insulin daily
- Diet adequate to support normal weight and activity
- Urine sugar free in majority of specimens
- Blood sugar levels never over 160 mg. fasting or 250 mg. postprandial (Folin-Wu)
- No history of acidosis, or frequent or severe hypoglycemic reactions
- Good peripheral circulation
- No retinopathy, electrocardiographic abnormality or abnormality of X-ray findings
- Regular medical supervision, at least once a year.

The above requirements of the Fidelity Mutual Life Insurance Company are reasonably representative of general insurance practice today but will differ in some details in various companies. Neither clinical nor insurance opinion is fully stabilized as to factors affecting the diabetic patient's length of life.

During recent years, 35 per cent of our diabetic applicants have satisfied the above very selective criteria. An additional 36 per cent have still proved insurable on some basis because their departure from the basic criteria was not too great. Acceptable departures from basic requirements have chiefly been questionable control as evidenced by mild hyperglycemia or moderately excessive glycosuria, need of more than 60 units of insulin daily, diabetes duration exceeding twelve years, excessive weight or a blood pressure slightly above 140 systolic or 90 diastolic.

Diabetic applicants with marked departures from the above criteria have been considered uninsurable. In our recent experience, 29 per cent of diabetic applicants have proved uninsurable on any basis, chiefly because they have been found to be under obviously poor medical control, or have had frank hypertension or other impairment in addition to the diabetes. Most commonly, the associated impairments have been vascular.

DATA ESTABLISHING ORIGINAL DIAGNOSIS

Data on which the original diagnosis of diabetes has been based are important and can influence the basis

of insurability in the borderline case. A negative definition best serves brevity. For insurance purposes, our office considers an applicant nondiabetic if both his fasting blood sugar level and his two-hour level have remained below 120 mg. in venous blood by the Folin-Wu technic, a slightly higher level being permissible in two-hour capillary blood, by the Folin-Malmros method. With the Somogyi or similar method, these levels should not exceed 100 mg. With such blood sugar findings our office has disregarded glycosuria in any degree and has disregarded high peaks in the blood sugar curve.

An occasional applicant whom our company would consider as nondiabetic, as defined above, may be under medical observation because of glycosuria and a "high peak" in a sugar tolerance curve which is otherwise normal. Although many clinicians may consider such borderline cases as "potential diabetes" or "mild diabetes," insurance at standard rates is sometimes possible.

Detailed analysis of blood sugar studies in the clinical laboratory of the Fidelity Mutual Life Insurance Company has been previously recorded.² A later study by this company, on policies issued from 1935 to 1958, followed to policy anniversary in July 1959, covered 4,966 years of exposure on such normoglycemic glycosuria cases. All of these policies were issued at standard premium rates. During that quarter century, there were twenty-three deaths in this normoglycemic glycosuria group, whereas we would have expected a fraction over thirty deaths among a similar number of the general class of standard insured policyholders of same ages during this period. The fact that the mortality of these normoglycemic glycosuria cases was somewhat more favorable than the mortality of standard policyholders in general was very probably because of more rigid selection of the glycosuria group in other respects.

COMMENT ON VARIOUS QUESTIONS RELATING TO INSURABILITY

Much of diabetes underwriting must rest on medical judgment. Insurance experience is still meager. Changes in empirical underwriting rules appear from year to year as experience grows. Many diabetic persons who were considered uninsurable not long ago are considered insurable now. Some classed as uninsurable now will undoubtedly be considered insurable in the future as careful observation, recording and study replace clinical impression.

How soon after diagnosis is the diabetic patient insurable? Originally he needed to wait three years. Today, the patient with mild diabetes is usually consid-

ered insurable as soon as it is evident that he has come under good medical supervision and control. Sometimes that is almost immediately.

Does the length of time a person has had diabetes affect his insurability? Duration of diabetes affects the cost of insurance. Higher death rates have been anticipated among persons whose diabetes has existed more than ten or twelve years because of the high incidence of complications shown by numerous clinical studies. One of the more recent of such studies is that of MacNeal and Rogers³ who in 103 diabetic patients found 53 per cent with retinopathy, 37 per cent with some heart impairment, 12 per cent with impaired PSP excretion, 8 per cent with albuminuria and 32 per cent with peripheral vascular disease. Barker's⁴ recent observation of insured lives, however, suggests that past duration of diabetes may influence death rate among diabetic persons less than has been anticipated, when absence of complications has been demonstrated by careful medical study.

Are juvenile diabetic patients insurable? Not today, in most companies. Our office has discouraged applications from diabetic persons under fifteen years of age, although some companies set age ten as the lower limit. The writer has reviewed most of the medical records of youthful campers at the Pennsylvania Camp for Diabetic Children. Records here from 1936 to date indicate that not many with juvenile diabetes can meet present insurability criteria as related to degree and stability of control. Of diabetic persons who have completed applications for insurance, 56 per cent below age twenty have proved insurable; at age twenty to twenty-nine, 67 per cent; at age thirty to thirty-nine, 86.5 per cent; at age forty to forty-nine, 67 per cent; at ages above fifty, 71 per cent; at all ages, 71 per cent. At young ages one meets the frequent problem of diabetes which is severe; at older ages one meets the frequent problem of vascular degeneration.

Does the amount of insulin needed by the diabetic patient affect his acceptability? Amount of insulin needed is considered an index of severity of diabetes. At first only those who could be controlled with 50 units or less daily were considered acceptable. Today, 75 units is a common upper limit for acceptance but patients needing over 50 or 60 units must usually pay a slightly larger premium for insurance.

What is the attitude of insurance companies toward diabetic patients who are being treated with the newer oral preparations? Opinion in insurance circles, like clinical opinion, differs widely. Some insurance medical officers⁵ have hesitated to consider orally treated pa-

tients acceptable on any basis. Some have considered them insurable but not eligible for so favorable a premium as patients controlled by insulin. In our office we have considered patients well controlled by diet alone as the most favorable of all, with patients well controlled by phenformin hydrochloride, tolbutamide, chlorpropamide or similar preparation, and patients controlled by small amounts of insulin in about the same category.

What is meant by "good control," for insurance purposes? The diabetic patient is considered under good control if he is on a well-balanced diet adequate to support normal weight and activity, voids specimens which are sugar-free most of the time and has shown periodic blood sugar levels not above 160 mg. fasting or 250 mg. postprandial by the Folin-Wu method. True blood sugar levels should be about 20 mg. lower. Degree of control is unknown in patients who rarely report to their physicians and such persons must be considered uninsurable. In uncomplicated diabetes, knowledge of good control is the most essential requirement for risk acceptance. Our office has examined a number of otherwise intelligent diabetic applicants who have been overconfident in self-control of diabetes and who at examination have revealed 3 per cent or more of glucose in a random urine specimen, with two or three hour postprandial blood sugar of 300 mg. or more. A favorable influence might be exerted on patient and physician alike if no diabetic patient were accepted for insurance unless he has habitually reported to his physician at intervals not exceeding six months.

Are patients with vascular complications insured? Evidence of vascular disease, such as absent dorsalis pedis pulses, vascular changes in eye grounds, and urinary findings suggesting nephropathy render the diabetic applicant uninsurable. While there has been a recent trend toward acceptance of some with slightly abnormal vascular findings, such acceptances are exceptional. Rigid requirements as to healthy blood vessels persist in spite of the fact that certain changes, such as demonstrable calcification of peripheral vessels, can sometimes be compatible with long life. A diabetic officer of our company, for example, had an X ray of lower leg because of injured ankle at age sixty-five in 1932. X ray revealed marked arterial calcification. This officer rarely missed a day of work, retired at age seventy-eight and died at age eighty-five, twenty years after frank calcification was demonstrated. The symptom of intermittent claudication did not appear until a few years before death. Diabetes was known to exist for over twenty years but record fails to show date of

onset. Although many clinicians can, from their records, exemplify similar long survivals with peripheral calcification, the individual with such findings is not today considered an acceptable risk for insurance.

How important are the electrocardiogram and X ray of the heart and lungs in diabetic risk appraisal? Our office does not require such studies of the diabetic applicant unless he is over fifty years of age, has had diabetes for over ten years or is applying for over \$50,000 of insurance. Findings of prior electrocardiographic and X ray studies have frequently been included in reports from attending physicians. Only rarely have findings been reported as abnormal, probably because 62 per cent of our diabetic applicants have been under age forty and because over three fourths of them have had diabetes ten years or less. The high incidence of coronary deaths noted by Barker⁴ and others suggests that companies which require an electrocardiogram almost routinely may be wise. Chest X ray may be a little less essential. Tuberculosis is rare in the economic class represented by insurance applicants. Abnormality of heart and great vessels, as revealed by X ray, is perhaps not much commoner among diabetic persons than among the nondiabetic, at identical ages. The writer⁵ some years ago measured transverse diameters of heart and of frontal aortic silhouette in photofluorograms of 1,053 diabetic patients of Philadelphia clinics. These diameters were compared at various weights and ages with similar measurements on photofluorograms of 1,000 nondiabetic clinic patients. Thus analyzed, incidence of X ray abnormality of heart and aorta was not remarkably greater among diabetic than among nondiabetic patients, when age and build were identical. Photofluorographic technic, in the review mentioned, was inadequate for reliable study of aortic calcification.

What is the attitude of the insurance medical officer toward diabetic applicants on "free diet?" Such applicants generally fail to meet life insurance criteria for good diabetes control, if we define this therapeutic method as weight control with absence of ketonuria, disregarding hyperglycemia and disregarding degree or constancy of glycosuria. Weight control alone provides adequate diabetes control for some patients and these would be considered insurable, regardless of therapeutic terminology applied. Conservative medical management, as opposed to the "free diet" hypothesis, has been evident among most of our diabetic applicants.

LIFE EXPECTANCY AND DEATH RATES OF DIABETIC PERSONS

Life insurance experience has been of relatively short

duration and information concerning long-term prognosis in diabetes is, therefore, available only from clinical sources. The latest of a series of statistical studies of Joslin Clinic patients by the Statistical Department of the Metropolitan Life Insurance Company⁷ was released in 1957. This analyzed experience for the period 1947 to 1951 on patients first treated at the clinic in the years 1930 through 1951. The diabetic person at age ten was found to have a life expectancy of 44.3 years compared with 61.5 years at age ten in the general population; at age twenty, 36.1 years compared with 51.9 years; at age thirty, 30.1 years compared with 42.5 years; at age forty, 23.7 years compared with 33.3 years; at age fifty, 16.9 years compared with 24.7 years; at age sixty, 11.3 years compared with 17.2 years. In this study, diabetic life expectancy was almost three fourths of normal at ages ten to fifty; about two thirds of normal at age sixty.

Death rate among diabetic patients, as observed in the same study, was seven times the death rate in general population at age twenty; about ten times at age thirty; almost four times at age forty; about two and three-quarter times at age fifty, and about two and two-third times the death rate of the general population at age sixty.

In the above analysis, it must be remembered that the recorded death rates among clinic patients were among them all, with or without complications and regardless of severity or duration of diabetes or its degree of control. Diabetic persons accepted for insurance are without serious complications and are under adequate control. Mortality assumptions used by insurance companies are, therefore, based on expectation of lower death rates than those shown in the above study of clinic patients.

MORTALITY STUDIES OF INSURED DIABETIC PERSONS

Mortality studies of insured persons with diabetes must at present be considered as reflecting short-term prognosis. Sizable insured groups have only recently become available for observation and study. Published insurance experience is still meager. Studies to date, however, suggest that among carefully selected diabetic patients, meeting criteria outlined in this discussion, the mortality in early years after issuance of insurance may be relatively favorable, not very far above the mortality of the general body of standard insured policyholders. This is to be expected, as it is the vascular complications in diabetes that cause most of the deaths and these complications do not become common among diabetic patients until they have had diabetes for ten years

or more. The earliest insurance experience is Montgomery's 1947 review⁸ of 373 diabetic policyholders of the Manufacturers Life Insurance Company of Canada, with one death, the expected mortality not being stated. Montgomery later¹ estimated that diabetic policyholders of this company were showing a death rate from one and one-half to two times that of the company's general class of standard policyholders and commented that this experience was heavily weighted with recently acquired cases. At the time of this later review, the Manufacturers Life had insured 550 persons with diabetes, thirteen of whom had died.

The Fidelity Mutual Life Insurance Company began insuring persons with diabetes about ten years ago. This company's experience is too short and too small to be meaningful from the statistical standpoint but to date reflects the rather favorable mortality picture that can occur in the early years after patients have met standards of insurability. With an insurance exposure of 581 policy years, there have been three death claims, approximating one and one-half times the number expected among an equal number of this company's standard policyholders of same age and during the same period of exposure. Coronary occlusion caused all three deaths.

Barker⁴ in 1959 summarized mortality experience of the Connecticut General Life Insurance Company as related to 777 policies issued to diabetic persons in the years 1946 through 1957, followed to policy anniversaries in 1958, and involving ten million dollars of insurance. Average duration per policy was five years, a short exposure for study of prognosis in diabetes. Seventeen persons with diabetes insured under twenty-three policies died during the period reviewed. The death rate among the diabetic insured was one and three-quarter times the death rate of this company's standard insured policyholders of similar age during the same period.

The foregoing relates to the entire class of diabetic policyholders. A favorable subgroup, including 468 policies and 60 per cent of all diabetic cases insured, was separately studied. Criteria for these include normal chest X-ray and electrocardiogram in all cases and in other respects approximate criteria of the Fidelity Mutual, described earlier. These, the most favorable cases, revealed only three deaths in the same period and a death rate actually lower than that of standard policyholders in general. This portion of the study again illustrates the tendency for mortality to be relatively favorable in the early years after diabetic persons have met basic insurance requirements. It also suggests how

vital to prolongation of diabetic life is good weight control, conservative medical supervision and a diligent attitude on the part of the patient in following adequate medical advice.

Among the remaining 309 diabetics accepted for insurance not in this favorable class, chiefly because of moderate overweight, moderate elevation of blood pressure, larger insulin requirements, glycosuria, or inadequate dietary control or medical supervision, the death rate was over four times the death rate of standard policyholders in general.

Of the seventeen deaths in the entire study recorded by Barker, coronary heart disease caused twelve, and cerebral hemorrhage and diabetic acidosis one each.

SUMMARY

Persons with diabetes were first able to procure insurance on this continent in 1940. In the early stages of appraising such risks for life insurance, only one in five met requirements for acceptance. Today, almost three fourths of adult diabetic applicants are found to be insurable. Early diagnosis and treatment, resulting in part from over a decade of publicity and effort related to the annual Diabetes Detection Drive, have undoubtedly augmented this favorable trend. Presently anticipated mortality among insured diabetic lives is only a fraction of the mortality observed in the past among unselected clinic patients. Present insurance rates reflect this more favorable prognosis. Insurance mortality studies have to date justified the optimistic prognostic prediction regarding patients whose diabetes is diagnosed early, is uncomplicated and is under conservative medical control.

SUMMARIO IN INTERLINGUA

Le Assecurabilitate de Personas con Diabete Mellite

In iste continente diabeticos poteva obtener assecurantia le prime vice in 1940. In le prime tempores del evaluation del riscos in le assecurantia del vita, solmente un applicante in cinque poteva satisfacer le requirimentos pro le acceptance. Hodie quasi tres quartos del adulte applicantes diabetic es considerate como assecurabile. Iste tendentia favorabile sin dubita ha essite accelerate per precoce diagnose e tractamento, le qual resulta in parte de plus que un decennio de publicitate e effortio relative al annual Campana de Detection de Diabete. Al presente le mortalitate expectate inter diabeticos assecurate es solmente un fraction del mortalitate observate in le passato inter non-seligite patients de clinica. Le presente premios de assecurantia reflecte ille plus favorabile prognose. Studios de mortali-

tate assecurantal ha usque nunc justificate le prognose optimistic in re le patiente con un diabete diagnosticate de bon hora e sin complication, qui es sub tractamento medical conservatori.

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Two aspects of the composition of the diabetic diet continue to receive attention. The first is the effort to provide carbohydrate in a form which can be better utilized by the diabetic than glucose. Fructose is known to be metabolized by the liver in the diabetic state at essentially normal rates. The comparatively rapid rate at which fructose is converted to glucose by liver, intestine and perhaps other tissues in the diabetic makes the added expense of substituting fructose for glucose hardly practical. The possible usefulness of sorbitol in diabetic diets was first suggested by Thannhauser in 1929. Although this sugar alcohol enters the metabolic processes of the liver by conversion to fructose, its slow rate of intestinal absorption is advantageous in diabetes (*Nutrition Reviews* 14:236, 1956; W. H. Olmsted, *Diabetes* 2:132, 1953).

The blood sugar changes which occur in diabetic patients when eating 200 gm. of an ice cream containing 36 gm. of sorbitol have been reported by C. R. Shuman, R. L. Kemp, R. Coyne and M. G. Wohl (*Am. J. Clin. Nutrition* 4:61, 1956). In mild or moderately severe diabetic patients the feeding of sorbitol ice cream as addition to the usual diet did not significantly alter the diurnal blood sugar in some patients with severe diabetes. E. P. Joslin (*Treatment of Diabetes Mellitus*, Tenth Edition, Lea and Febiger, Philadelphia, 1959) comments favorably on the use of sorbitol as a glucose substitute and sweetening agent in diabetic diets. Sorbitol would appear to have promise in providing slowly absorbed carbohydrate between meals and at night for

individuals prone to hypoglycemic attacks.

The second aspect of the diabetic diet which has received current attention concerns the possible harmful effect of fat in the diabetic diet. The suspicion is widespread that the diets with a high proportion of calories as saturated fatty acids formerly advocated in diabetes may have promoted the development of atherosclerosis. The enhanced frequency with which diabetic patients fall victim to many of the complications of atherosclerosis makes the possible role of diet in this process of great significance. The recent experimental findings demonstrating a cholesterol lowering effect of unsaturated fatty acids of the linoleic type (*Nutrition Reviews* 15:1, 1957) suggests that this information be translated into recommendations concerning the sources of fat permitted a diabetic patient. However, direct evidence of the protective effects of unsaturated fatty acids upon the development of the atherosclerotic lesion in man is still lacking, making major qualitative shifts in fatty acid intake unjustified.

Measures which achieve a moderate increase in unsaturated fatty acids without altering the type or taste of foods in the present menu can have little objection. This limited goal can be achieved by substituting certain vegetable oils and margarines of high content of unsaturated fatty acid for saturated fats widely used at present. It is hoped that clarification of this important question will soon be forthcoming.

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Recent Statistics on Diabetes

The mortality from diabetes in the United States in the first half of 1960 was about 7 per cent higher than in the corresponding period of 1959, according to provisional figures based upon a 10 per cent sample of the death certificates. The major part of this increase occurred in the early part of the year, with an 11 per cent increase during the first quarter as compared with one of only 3 per cent in the second quarter over the corresponding periods of 1959. This trend follows the pattern of the mortality from all causes which, in the first quarter of the year, showed an increase of 6 per cent over 1959, but in the second quarter, a slight decrease from 1959. An important factor in the situation was a relatively high prevalence, in early 1960, of respiratory disease which so often is associated with a rise in mortality among persons with chronic disease.

The rise in diabetes mortality in the country as a whole in this recent period is found also among urban dwellers, as represented by the experience among Industrial Policyholders of the Metropolitan Life Insurance Company.

In most of the limited area for which mortality reports are regularly received, the mortality from diabetes has risen, but small decreases were reported for Baltimore and Boston as shown in table 1.

In Canada, as so often happens, opposite trends are reported for Toronto and Montreal, the former showing a moderate increase and the latter a substantial decrease in the first half of 1960 as compared with 1959. As for England the only figures available, for London Administrative County, show a moderate increase.

Provisional figures for all of England and Wales are now available for the year 1959. They show a slight falling off from 1958, with a decline of much the same degree for males and females.

Comparison of regional data for the United States on diabetes mortality for the first half of 1960 with those for the first half of 1959 is given in table 2 which shows fairly sizable increases for all areas ex-

Submitted by the Committee on Statistics, Herbert H. Marks, Chairman. The Committee welcomes suggestions or actual materials suitable for this section in future issues from Association members and other readers of the Journal.

TABLE 1

Recent data on diabetes mortality
Deaths and death rates—January-June 1960 and 1959

Area	Death rates per 100,000		Number of deaths	
	1960	1959	1960	1959
United States (10% sample)	17.9	16.7	1,591	1,452
Metropolitan Life Insurance Company Industrial Policy- holders	16.5	16.1	1,320	1,311
New York State	19.2	18.6	1,617	1,530
New York City	21.4	19.6	859	787
Maryland	18.9	18.2	291	274
Baltimore	24.2	24.6	119	120
Boston	16.7	17.7	57	61
Philadelphia	22.2	19.0	244	209
Toronto	19.3	17.9	63	58
Montreal, resident	13.7	15.6	81	91
London (Administrative County)	8.9	8.1	142	130
	1959	1958	1959	1958
	(Jan.-Dec.)	(Jan.-Dec.)	(Jan.-Dec.)	(Jan.-Dec.)
England and Wales				
Total	7.0	7.4	3,193	3,316
Males	5.0	5.3	1,100	1,153
Females	8.9	9.3	2,093	2,163

NOTE: Rates for the states and cities are based upon local estimates of population. United States data based upon the returns from a 10 per cent sample of death certificates received in vital statistics offices, as published in "Current Mortality Analysis," a monthly report of the National Office of Vital Statistics of the U. S. Public Health Service.

cept the Northeast and the Pacific Coast states, where there were decreases of only minor degree.

The variation in mortality from diabetes according to marital status is a subject of prime interest. Detailed statistics of this kind are available for the period 1949-51 when population figures necessary for computation of death rates could be obtained from the national census. Such comparisons relate to age-specific data because of the differing age composition of the various categories according to marital status. In reviewing the results it must be kept in mind that a significant degree of misstatement on marital condition exists both in census returns and death certificates, particularly for the widowed and divorced. While it would be preferable to have broader categories for comparison—namely, those ever married and those never married—data on this basis are not available.

As table 3 shows, white married men at ages up to

RECENT STATISTICS ON DIABETES

TABLE 2

Number of deaths and death rates from diabetes in geographic division: United States reporting area for the 10 per cent sample—January-June 1960, 1959 and 1958

Geographic division	Death rates per 100,000*			Number of deaths*		
	1960	1959	1958	1960	1959	1958
U. S. reporting area	17.9	16.7	16.3	1,591	1,452	1,384
New England	19.8	20.3	22.4	97	96	101
Middle Atlantic	23.5	23.7	20.4	400	391	333
East North Central	19.6	18.7	19.5	362	335	343
West North Central	20.2	17.8	15.5	155	137	118
South Atlantic	14.1	12.3	15.1	183	156	188
East South Central	15.0	13.1	13.1	90	78	77
West South Central	17.5	14.8	12.5	147	123	102
Mountain	11.0	8.4	9.7	37	28	31
Pacific	11.8	11.9	10.1	120	108	91

*Excludes armed forces overseas.

NOTE: These data from the 10 per cent sample are subject to sampling error. The number of deaths, as given, does not cover the entire United States for each month but is limited by the completeness of the reporting area. The size of the reporting area is indicated by the footnote on page 7 of each monthly issue of the "Current Mortality Analysis."

SOURCE: Data furnished by National Office of Vital Statistics of the U. S. Public Health Service.

seventy experienced lower rates than single men, but at ages seventy and over the reverse holds. The difference between the two groups was proportionately greatest at ages under forty-five. The recorded rates among the widowed and divorced were generally higher than among both the married and the single. For women the pattern of mortality according to marital status, was similar to that for men at ages under forty-five, but at later ages the married women had consistently higher rates than single women. The margin between the two was quite large and tended to increase both absolutely and relatively with age. Between ages sixty and

seventy-four the rates for married women were more than double those for single women. Rates for widows tended to be slightly higher than among the married whereas among the divorced they were appreciably less at most ages.

The higher rates among single persons at the younger ages reflects the lower marriage rates among young diabetics as compared with nondiabetics. As for the differences in middle and later life several factors are at work, although these are not identical for the two sexes. Single men who become diabetic, particularly at the working ages, are less likely and less well motivated than married men to seek and follow treatment. In part, this may reflect socio-economic differences between the single and the married. This is less true among women. Moreover, middle-aged single women, particularly if they are employed, are more likely to control their weight. Probably of even greater importance with respect to the high rates among married women are endocrine factors relating to pregnancy and childbearing.

While much interest attaches to differentials in diabetes mortality according to occupation and socio-economic status, the data on this subject are not entirely adequate. As in the case of marital status, statements about occupation in the census returns and on death certificates do not always correspond to each other. A tendency to "upgrade" the occupation exists in both sources of data, but more especially on death certificates. Furthermore, deaths from diabetes during the period of active working life include only a minority of all deaths from the disease, particularly in the higher socio-economic groups. A distinct source of bias is that persons with relatively poor physique or who acquire handicapping disease, tend to seek employment

TABLE 3

Death rates per 100,000 from diabetes mellitus by marital status by race, sex and age. United States, 1949-1951

Age groups	White males				White females			
	Single	Married	Widowed	Divorced	Single	Married	Widowed	Divorced
20-24	1.3	0.5			1.6	1.1		
25-34	4.9	1.3		6.5	3.9	1.5		3.5
35-44	9.6	2.8		12.6	4.7	2.5	4.2	4.2
45-54	17.5	8.9	19.7	19.6	9.4	11.5	16.1	10.5
55-59	30.0	22.6	31.1	33.2	20.9	38.1	43.5	33.5
60-64	45.9	38.8	52.7	53.8	32.2	74.7	81.2	65.0
65-69	63.8	60.8	78.9	84.6	47.3	111.6	120.7	86.9
70-74	85.6	95.6	108.4	124.4	71.9	162.1	163.2	170.8
75+	112.9	139.9	153.5	182.9	114.5	193.9	195.4	269.5

NOTE: Rates are not shown in age groups with less than twenty deaths.

SOURCE: National Office of Vital Statistics, Vital Statistics-Special Reports, Selected Studies, Vol. 39, No. 7, May 8, 1956.

in the lighter occupations which, by the definitions used, often rate somewhat higher in the socio-economic scale than those involving manual labor.

The best recent data on the subject are based on mortality rates for England and Wales during 1949-53, the five-year period centered around the last census there in 1951. Table 4 gives the facts for diabetes, as

TABLE 4
Mortality from diabetes mellitus by social class
Ages 20-64, England and Wales, 1949-1953

Social class	Standard mortality ratios		
	Men	Women Married* Single	
I Professional, managerial, etc., occupations	134	60	†
II Intermediate	100	80	78
III Skilled	99	100	82
IV Partly skilled	85	122	77
V Unskilled	105	119	75

*Classified according to husband's socio-economic status.

†Less than ten deaths.

SOURCE: The Registrar General's Decennial Supplement, England and Wales, 1951. Occupational Mortality, Pt. II, Vol. I, Commentary, Her Majesty's Stationary Office, 1958.

TABLE 5
Mortality from diabetes mellitus in selected socio-economic groups or social subclasses, Ages 20 to 64, England and Wales—1949-1953

Socio-economic group or social subclass*	Social class	Standard mortality ratio	
		Men	Married women†
Higher administrative, etc.	I	134	60
Clerical workers	III	126	75
Shopkeepers	II	124	70
Armed forces—noncommissioned men	III	123	133
Personal service	III	120	85
Shop assistants	III	113	79
Unskilled workers	V	104	119
Transport workers	III	100	113
Farmers	II	98	127
Skilled workers	III	95	107
Other administrative, etc.	II	91	72
Drivers of goods vehicles	III	89	—
Builders, laborers	V	86	95
Foremen	III	86	85
Agricultural workers	IV	82	131
Building and dock workers	V	81	100
Semiskilled workers	IV	79	124
Mineworkers	III	67	205

*Classified according to occupation.

†Classified according to husband's socio-economic status.

SOURCE: The Registrar General's Decennial Supplement, England and Wales 1951, Occupational Mortality, Pt. 11, Vols. I and II, Her Majesty's Stationary Office, 1958.

‡Number of observed deaths in each classification expressed as a percentage of the number expected on the basis of the age-specific mortality for the sex in the general population.

recorded in the death returns, in the form of Standard Mortality Ratios‡ for males, married women and single women according to social class. Table 5 gives similar data for the males and married women for certain subdivisions of the social class groups. In these tabulations married women are classified according to the socio-economic status of the husband.

Among males the mortality ratio was highest in Class I, comprising the professional, managerial and propertied groups, and lowest in Class IV which consists primarily of semiskilled workers. The ratios for males in the remaining three classes did not vary much from the average for all males. In contrast, married women showed significantly lower ratios in the upper socio-economic classes than in the other classes and a steady increase in the ratios from Class I to Class IV. The ratio for Class V was not significantly different from that in Class IV. Among single women the variations with social class were relatively minor.

The meaning of these variations is far from clear. The substantial equality of the figures in three major classes among the males may, to some extent, reflect the general leveling of living conditions. The high ratio in Class I may be due, in part, to the high proportion in sedentary occupations and in those which encourage overeating and thus obesity. The results may also reflect the inclusion of deaths of men in Class I whose true occupational status is Class II or lower. On the other hand, the low ratio in Class IV may reflect, in part, occupational selection and also, in part, upgrading of occupations which are actually Class V. The trend of mortality by social class among married women is believed primarily to reflect greater attention to personal health on the part of those in better financial and social circumstances, including more regular and frequent medical care. This would in turn be reflected in a longer duration of life of those becoming diabetic and perhaps a low frequency of diabetes through prevention of obesity.

In the subdivisions by socio-economic and social subclasses, high ratios are recorded for men in higher administrative jobs (Social Class I), clerical workers (III), shopkeepers (II), personal service workers (III), shop assistants (III), and noncommissioned men in the armed forces (III). Low ratios are found among mine workers (III), semiskilled workers (IV), building and dock workers (V), agricultural workers (IV), and foremen (III). Interesting contrasts are found in the high ratios for wives of mine and agricultural workers, occupational groups in which men have low ratios. Differences in the opposite direction are found for

TABLE 6

The causes of death among 325 diabetic patients dying in 1959-60,* by sex: Experience of Joslin Clinic

Cause of death	Number of deaths		Per cent of all causes	
	Males	Females	Males	Females
All causes	150	175	100.0	100.0
Diabetic coma (primary)	1	2	0.7	1.1
Cardio-renal-vascular	110	133	73.3	76.0
Arteriosclerotic	110	131	73.3	74.9
Cardiac	64	88	42.7	50.3
Coronary	45	60	30.0	34.3
Renal	25	15	16.7	8.6
Diabetic nephropathy	18	10	12.0	5.7
Cerebral	18	22	12.0	12.6
Gangrene	1	3	0.7	1.7
Site unassigned	2	3	1.3	1.7
Other circulatory and rheumatic disease	—	2	—	1.1
Infections, total	12	10	8.0	5.7
Pneumonia and respiratory	7	5	4.7	2.9
Gall bladder	1	—	0.7	—
Kidney, acute	1	3	0.7	1.7
Other infections	3	2	2.0	1.1
Cancer	11	24	7.3	13.7
Accidents	3	1	2.0	0.6
Suicides	—	1	—	0.6
Insulin reactions	1	1	0.7	0.6
Other diseases	12	3	8.0	1.7
Average age at death (years)	61.5	65.4		
Average duration of diabetes (years)	16.2	16.0		

*Deaths reported from Jan. 6, 1960, through June 29, 1960.

wives of clerical workers, administrators, shopkeepers, shop assistants and personal service workers.

Recent deaths among diabetic patients of the Joslin Clinic have been reviewed to ascertain differences according to sex in the distribution of deaths by cause. The facts are shown in table 6. It is notable that in the aggregate deaths from cardiovascular-renal diseases formed approximately the same proportion—about three fourths—for each sex, with all but a small proportion classified as of arteriosclerotic etiology. The internal composition of this broad category showed rather significant differences in that the proportion with heart disease for females is somewhat the higher; this applies specifically also to coronary artery disease, whereas for males, the proportion of renal deaths and more specifically those ascribed to diabetic nephropathy was substantially the larger. The higher proportion of cancer deaths among diabetic women compared with men is similar to the situation in the general population.

In interpreting the data it should be kept in mind that the differences found pertain to the proportional mortality and not death rates. Moreover, the average age at death of the females is the higher. The average duration of diabetes in the aggregate is about the same in the two sexes, but when age groups at onset are compared, the figures for the females tend to be the higher.



EDITORIAL

HISTOLOGY OF SMALL BLOOD VESSEL DISEASE IN DIABETES

Clinicians and pathologists long have known that diabetic patients, usually after long duration of the disease, show morphologic changes in the arterioles, capillaries and venules which are seldom seen in nondiabetics. Fairly specific clinical syndromes are known to accompany these changes. These lesions and their

consequences commonly occur in addition to (and at times independently of) the ordinary atheromatous and arteriosclerotic lesions seen in the larger vessels of diabetic and nondiabetic patients alike.

Intercapillary glomerulosclerosis (Kimmelstiel-Wilson) and diabetic retinopathy caused by small vessel disease are familiar to most physicians. So are the coronary, cerebral, renal and peripheral vascular complications presumed to be caused by lesions of larger vessels. Recent pathological studies suggest that the confusion and uncertainty concerning these two types of vascular disease and their consequences may soon be lessened.

If the lesions in the retinal vessels and glomeruli so characteristic of long-standing diabetes are in fact related to diabetes, it is strange that they have not been found in other organs. There is no doubt that they can and do occur without ordinary arteriosclerosis, but they have seemed to be limited to the eye and kidney. Ditzel¹ has reported that conjunctival vessels also show

characteristic changes in diabetics as well as in their nondiabetic children.

Evidence recently accumulated by Blumenthal, Goldenberg and colleagues in St. Louis now sheds some light on the vexing questions of distribution and specificity. In 1957 this group described² an obliterating endarteritis, similar to that which is well known in other organs, in the arteries of the placenta in diabetic mothers. More recently these investigators reported interesting new findings in the blood vessels of the extremities of diabetic patients.³ The observations make it appear that "diabetic angiopathy," as it is often called abroad,⁴ may be specifically related to diabetes, and that it appears in many organs besides the kidneys and eyes.

One hundred and fifty-two amputated extremities were examined,⁵ with special attention to vessels smaller than the digital arteries. Ninety-two extremities came from diabetic patients and sixty from nondiabetics, one half with obliterative vascular disease. Differential stains were employed including the familiar periodic acid Schiff, and the less well-known Rinehart modification of a stain introduced by Hale, by which certain acid mucopolysaccharides are seen to have an unusual affinity for colloidal iron.⁶

The differences between the small vessels in the diabetic and nondiabetic patients were so clear that the investigators, without knowing the source of the material, were able to identify the specimens from diabetic subjects with a remarkable degree of accuracy (eighty-five out of ninety-two). The specimens from nondiabetics were recognized equally well (fifty-seven out of sixty). The greatest histological contrasts were found in the endothelium of the small arteries. The specimens from diabetics characteristically consisted of marked proliferation of swollen endothelial cells, frequently obliterating the lumen, in contrast to the flattened endothelium, marked fibrosis and hyalinization so common in arteriosclerosis. In diabetes the proliferated endothelium was interlaced with PAS-positive hyaline fibrils which failed to take the colloidal iron stain. Identical differences in staining were present in the hyaline material in the glomerular tufts in "diabetic glomerulosclerosis." In contrast, arteriosclerotic vessels containing hyaline and fibrotic changes characteristically stained positively with colloidal iron and not very well with PAS. The internal elastic membrane in the small vessels of the diabetics was intact and it stained strongly with PAS, whereas in arteriosclerosis it was frayed and reduplicated and did not stain well. The media in the sclerotic vessels contained more cal-

cium and bone, was more hypertrophied, and usually contained less PAS-positive material than in the diabetics. When hypertension existed these differences were less striking.

Similar staining characteristics were found in diabetics, also, in the vasa vasorum, vasa nervorum, small dermal vessels and arterioles in the muscle septa. A remarkably close correlation existed between the lesions in the vasa nervorum of the extremities of diabetics and antecedent clinical signs of neuropathy, suggesting that the angiopathy was responsible for the neurologic disease, as has been postulated by others.⁸

More recently the histology of the coronary arteries of 116 autopsied diabetic patients was compared with that of 105 nondiabetics.⁷ Less consistent differences were found in the small intramural branches than in the arteries of the extremities. Even so, proliferative endothelial lesions were from two to two and one-half times as frequent in the diabetic as in the nondiabetic subject.

A surprising product of the studies by this group is the suggestion that arteriosclerosis of major arteries in diabetes may be no higher in incidence or intensity than in nondiabetics, if factors of age and coexistent hypertension are properly assessed. If this observation is correct (it has been confirmed by others) the characteristic form of small vessel disease in diabetes may be a greater culprit in complications like patchy gangrene, "arteriosclerotic" heart disease, neuropathy, skin lesions, and other common diabetic complications than previously supposed. It is pointed out that in the small vessels obliterative changes may make it less easy to develop collateral circulation as occlusion of large vessels occurs.

Although histochemical findings provide a clue in differentiation, pathological studies alone cannot reveal the precise cause of the angiopathy. It is presumed to be metabolic, or perhaps immunologic, in origin and thus distinct from the lipid, fibrous, calcific and muscular changes seen in hypertensive vascular disease and arteriosclerosis. The latter types of disease are thought by many pathologists to be primarily mechanical, not chemical, in origin.

Interested students of vascular disease in diabetes must now await confirmation or denial of the observations of the St. Louis group. Not much time should be required for experienced pathologists to refute or acclaim these exciting reports. If confirmed, it may not be long before the pathogenesis and prevention of many disabling vascular complications will become better understood.

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BOOK REVIEWS

THE ARTERIAL WALL. Edited by Albert I. Lansing, A.B., Ph.D. \$7.50, pp. 259, *The Williams and Wilkins Company, Baltimore, 1959.*

This volume will be especially welcome to those followers of the literature on atherosclerosis who have sensed an over-emphasis on diet and the lipid constituents of the plasma. Certainly progress can be expected only if these factors are considered in relation to the substrate where the disease is manifest, namely the walls of the blood vessels.

In the words of the editor, himself a long-time student of blood vessels, the volume "represents an attempt to review the data that are currently available on the structure, function and chemistry of the major components of the arterial wall." This objective is accomplished with great success in a series of chapters written by qualified investigators. The subjects covered are the vasa vasorum, endothelium, smooth muscle, elastic tissue, collagen and ground substance, mucopolysaccharides, enzymes, lipid metabolism and metabolism in general, all considered as components of or in relation to the arterial wall. Except for the chapter on collagen and ground substance, all are clearly presented. It is interesting and stimulating to find that the vast majority of the many references at the ends of chapters are dated since 1950. Most of the material has to do with the normal artery, but there are some data on aging and a few on atherosclerosis. The information is often basic, largely biochemical, almost entirely "functional." In fact, as is suggested in the concluding chapter, this book heralds the transition from a descriptive anatomic unit to a dynamic approach to the blood vessel wall.

One is left with a sense of incompleteness and frustration, a feeling obviously shared by the editor and contributors, caused by the vast gaps in our knowledge which become apparent when the available information in a field is carefully and intelligently collected, as it is here. The recognition of these gaps, is, in fact, a major function of this important book, which will doubtless serve as a base for investigations of the blood vessels for years to come.

HUMAN NUTRITION AND DIETETICS. By Sir Stanley Davidson, A. P. Meiklejohn and R. Passmore. \$15.00, pp. 844, *The Williams and Wilkins Company, Baltimore, 1959.*

This book, in common with most encyclopedic volumes, varies considerably from chapter to chapter. The attempt to write a book which would be useful to physicians and intelligible to nonmedical people has resulted at times in textual material that oversimplifies the medical aspect but still is too complicated for the nonclinically trained person.

The book is divided into six parts:

Part I, with the title of "Physiology," is rather more extensive than is usually found in this type of compendium. The discussion of fats and blood lipids is up-to-date. Much of the other material is at the medical student level and as such might appeal to the general practitioner.

Part II concerns itself with food and food composition, presented from the Food and Agriculture point of view.

Part III, on "Primary Nutritional Diseases," again is basically from the public health and epidemiological point of view rather than the practical day to day problems which the practitioner will encounter.

Part IV is "Nutritional Aspects and Dietetic Treatment of General Diseases." The terminology here is basically British, and the approach is that encountered more commonly by physicians who have received their training on the Continent. It does not conform entirely with the current teaching in this country.

Parts V and VI have to do with public health and diet in physiological stress.

To the American this book is written with a certain English whimsy and a delicious use of the English language not commonly found in American texts. It is not particularly suited for the clinician who is involved in the day to day problems of the office practice of medicine. It does, however, represent condensation of a tremendous amount of experience in international nutritional problems as encountered in underdeveloped countries in many parts of the world. From the point of view of those involved in this type of work, the book can be recommended very highly. It draws very extensively on the United Nations organizations, the World Health Organization and the Food and Agriculture Organization.

PYELONEPHRITIS. By Fletcher H. Colby, M.D. \$7.50, pp. 214, 94 illustrations, *The Williams & Wilkins Co., Baltimore, Maryland, 1959.*

The subject of this book has in recent years become one of great interest and importance not only to the urologist, obstetrician, pediatrician and internist but to all students of physiology and bacteriology. The author, therefore, has presented briefly the embryology, anatomy and physiology of the kidney as a preliminary to the presentation of clinical features. As a surgeon, he has stressed his long experience on the urologic service and gives many excellent roentgenograms. However, special chapters are devoted to pyelonephritis in infancy, pregnancy and in relation to diabetes and hypertension. The special responsibilities of the surgeon because of his use of traumatizing instruments and because of the difficulty of insuring cleanliness and asepsis are discussed in the concluding chapter. He contrasts the treatment of acute pyelonephritis, emphasizing the use of the newer drugs, with the greater difficulties and the manifold problems in the diagnosis and

treatment of chronic pyelonephritis.

This excellent book should prove useful and a welcome addition to the working library of many practicing physicians.

THE DIABETIC'S HANDBOOK. By Anthony M. Sindoni, Jr., M.D. \$4.00, pp. 285, The Ronald Press Co., New York, 1959.

In most illnesses it is unimportant for the patient to know what is going on. This is not the case with diabetes, where treatment rests in the hands of the patient himself, and where the physician acts only as guide and counselor, his most important task is that of educating the patient to carry out his own treatment correctly. For intelligent cooperation can come only with understanding of the nature of the disorder and a grasp of the rationale underlying treatment. To meet this need, many physicians have written guidebooks for the diabetic. Dr. Sindoni's handbook includes a large amount of useful data concerning diabetes, its complications, and its treatment. Over one hundred pages, for example, are devoted to discussion of the insulins, food values, and diet. Its faults are overinclusiveness and failure to confine itself to the diabetic patient. Much of the material is more appropriate to nurses, dietitians, or semiprofessional personnel having some background of medical knowledge.

Fifty-six of its pages are given to a chapter on "Complications of Diabetes," with numerous but superficial discussions by contributing authors on such subjects as "Cardiac (Heart) Surgery in the Diabetic Patient," "Anesthesia in Surgery for Diabetics," "Tuberculosis among Diabetics," "Kidneys in Diabetes," "Symptoms of Arteriosclerosis," and "Necrobiosis Lipoidica Diabeticorum." It is questionable whether it is productive of good to expose the patient to material which may increase his apprehensions.

These extra topics seem to have led to certain omissions. The discussion of insulin reactions does not touch upon the extremely important distinction between reactions of which the patient is aware and which he can handle without assistance, and those requiring assistance by others, nor is this mentioned in the section on employability of diabetics. Neither is there adequate discussion of the basic differences between stable and unstable diabetes and their effect on the management and the activities of the diabetic. The list of "Causes of Insulin Shock" omits gastric retention or vomiting, failure to ingest the prescribed amount of carbohydrate, improper carbohydrate distribution, and too long an interval between feedings. No mention is made of the need for afternoon interval feedings with the long-acting insulins, or of the need to adapt carbohydrate distribution to the type of insulin and to the patient's response curve. A one-page section on "Exercise" does not stress the importance of learning how much extra carbohydrate must be taken to compensate for extra physical activity. There is no discussion of leg exercises and walking in the treatment of circulatory insufficiency in the legs. The section on foot care omits the necessary precautions to be taken in cold weather by the diabetic with impaired peripheral circulation.

Finally, no book can discern, as the physician can (if he will take the time) the precise areas where the individual patient needs enlightenment, or the tactful care with which he

must be helped to face the facts of the diabetic life if he is to face them successfully. This is the function of the interested, experienced and understanding physician; no book can substitute. The real function of the guidebook is to provide a reference manual for the facts the diabetic needs to keep at hand—facts about foods and diet, about insulins, syringes and needles, materials and technics, test reagents and methods, the recognition and management of emergencies. Most of these matters are covered adequately in this book.

HORMONES AND ATHEROSCLEROSIS. Edited by Gregory Pincus. \$13.50, pp. 484, Academic Press, Inc., New York, 1959.

This volume represents a valuable compendium on the subject of its title. It consists of thirty-two papers, and discussions of these papers, from a recent symposium. Many of the contributors, several of them from foreign countries, have names already well known for research on atherosclerosis.

In general the book is well edited and well presented. A minor defect is the lack of a summary at the ends of most of the chapters; the reader is required to make a careful perusal of the text in each case. This lack is perhaps outweighed by the printing of an instructive discussion of each paper by other participants; in this way a healthy balance is achieved and any tendency to dogmatism is avoided.

The papers are well arranged, in that they begin with fairly fundamental discussions of cholesterol metabolism, pass through attempts to modify experimental atherosclerosis by the use of hormones (particularly represented by the school of Katz, Stamler and Pick), through efforts to modify the human disease by means of analogs or derivatives of thyroxine and estrogens, and end with a few philosophical speculations on emotions and stress. An interesting paper from South Africa indicates the lack of evidence that the relative immunity of the much-discussed Bantu to atherosclerosis has anything to do with hormones. One is left at the end with a (perhaps desirable) feeling of frustration and incompleteness, because there is so little solid fact and so many questions are left unanswered. For instance, diabetes is scarcely mentioned, and there is no attempt to explain the remarkable way in which diabetes tends to erase sex differences in the incidence of coronary occlusion.

Finally, one outstanding characteristic of this symposium is that fully twenty-nine of the thirty-two papers are devoted to some aspect of cholesterol metabolism or to the effect of hormones on blood levels of cholesterol or other lipids. Needless to say, this reflects the extraordinary, and (some would say) unfortunate preoccupation of most workers in the field with these substances. Only a partial counterbalance is achieved by the inclusion of a chapter on "The Arterial Wall as an Organ," and by a few statements such as the following concluding paragraph of one of the chapters: "As yet, there is no evidence that reduction of hypercholesterolemia in man is associated with inhibition or regression of the atherosclerotic process. It is therefore essential to assess any potentially satisfactory therapeutic regimen in terms of morbidity and mortality rather than in its ability to lower plasma cholesterol levels."

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Alterman, Seymour L. (Mount Sinai Hosp., Miami Beach, Fla.): DIABETES, ATHEROSCLEROSIS AND HYPERCHOLESTEREMIA: A PRELIMINARY REPORT ON THE STUDY OF DIETARY CONTROL. *Postgrad. Med.* 28:112-20, August 1960.

Since a definite relationship seems to exist between atherosclerosis and hypercholesteremia and since both these conditions often are prominent in diabetics, it would seem wise to keep the serum cholesterol of diabetic patients in a low, normal state. The main approaches to this are through diet, chiefly by substitution of polyunsaturated for saturated fats. The present diets of the American Diabetes Association contain too high a percentage of fat, and therefore have no lipid-lowering effect. W.R.K.

Armstrong, John R.; Daily, Ray K.; Dobson, Harold L.; and Girard, Louis J. (Jefferson Davis Hosp., Baylor University Coll. of Med., Houston, Tex.): THE INCIDENCE OF GLAUCOMA IN DIABETES MELLITUS: A COMPARISON WITH THE INCIDENCE OF GLAUCOMA IN THE GENERAL POPULATION. *Am. J. Ophth.* 50:55-63, July 1960.

The evidence presented suggests that the incidence of both primary and secondary glaucoma in diabetes is appreciably increased over that in the general population. This indicates that diabetics with any suspicious visual complaints should be considered for glaucoma testing, and that glaucoma patients with symptoms even mildly suggestive of diabetes be given routine sugar metabolism studies. W.R.K.

Arney, Glen K.; Pearson, Elinor; and Sutherland, Anne B. (U.S. Army Surg. Res. Unit, Brooke Army Med. Center, Fort Sam Houston, Tex.): BURN STRESS PSEUDODIABETES. *Ann. Surg.* 152:77-90, July 1960.

The syndrome of burn-stress pseudodiabetes occurring in two patients in the early post-burn course is presented in detail. In both instances the syndrome occurred during periods of balance study at which time a high-carbohydrate, high-calorie intake was being given. The clinical and laboratory manifestations of this syndrome are, in the order of appearance, hyperglycemia, glycosuria without acetonuria, high urinary specific gravity, and an intense osmotic diuresis. This results in severe dehydration with elevation of the nonprotein nitrogen, hemoglobin and hematocrit as well as a high serum Na and Cl. A marked increase in the urinary output is the most striking early clinical manifestation. The syndrome has been seen most frequently in patients who have a family history of diabetes mellitus. When recognized early it has been aborted by discontinuing forced feeding. If it becomes fully developed, appropriate therapy consists primarily of vigorous water replacement and sufficient amounts of insulin to control the hyperglycemia. The metabolic alterations pertaining to the etiology of the syndrome are discussed. W.R.K.

Baird, R. William; and Hull, John G. (Med. Sect., Hermann Hosp., Houston, Tex.): CHOLESTATIC JAUNDICE FROM TOLBUTAMIDE. *Ann. Int. Med.* 53:194-96, July 1960.

The author described the occurrence of cholestatic jaundice in a diabetic patient who had used tolbutamide. The diagnosis was made on a liver biopsy removed at operation. The patient had a background of impairment of liver function noted two

years before tolbutamide therapy. The latter was used intermittently and haphazardly in a dosage no larger than 3 gm. and for a period of about three months before the onset of jaundice. S.B.B.

Becker, Donald; and Miller, Max (Western Reserve Univ. Sch. of Med. and Dept. of Med., Univ. Hospitals of Cleveland, Cleveland, Ohio): PRESENCE OF DIABETIC GLOMERULOSCLEROSIS IN PATIENTS WITH HEMOCHROMATOSIS. *New England J. Med.* 263:367-73, Aug. 25, 1960.

The clinical records, autopsy reports, and sections of kidney stained with hematoxylin and eosin and PAS were reviewed in fifty-two patients with hemochromatosis. Of these fifty-two patients, twenty-two had associated diabetes. Of the twenty-two patients with hemochromatosis and diabetes, seven had diabetic glomerulosclerosis. Of these seven patients, four had both the diffuse and the nodular lesion and three had only the diffuse lesion. There were no cases of glomerulosclerosis in the thirty cases of hemochromatosis without diabetes. It is concluded that the diabetic glomerulosclerosis of ordinary diabetes mellitus also occurs in patients with diabetes and hemochromatosis. These findings stimulate the speculation that the development of diabetic vascular complications is in some way directly related to the metabolic defect, or an undiscovered common factor or factors that exist in all diabetic patients and eventuate in the specific vascular complications. W.R.K.

Bouton, Malcolm A.; and Cortesi, Joseph B. (Health Dept., City of Schenectady; and Ellis Hosp., Schenectady, N.Y.): A DIABETES CASE-FINDING PROGRAM. *Am. J. Pub. Health* 50:524-30, April 1960.

There was a prevalence of previously unknown cases of diabetes of nine per 1,000 (0.9 per cent) which may be slightly lower than should have been obtained. An occasional physician's reluctance to admit he was unaware that his patient was diabetic and reliance upon urinalysis and fasting blood specimens as diagnostic tools may also have affected the data. A diabetes case-finding program would be most profitable, if special attention were directed toward the screening of that segment of the population forty years of age and over. Fewer cases of diabetes will be missed if the screening test is done within one and one-half hours of an adequate meal. When the test is equivocal, the patient should be asked to return for retesting. Maximum sensitivity and specificity are obtained when the glucose screening level is at 160 mg. per 100 ml. The costs of the program are in the neighborhood of \$80 per new case found. The authors believe diabetes case finding to be a most worth-while local Health Department program in the area of chronic disease. W.R.K.

Chatterjee, I. B.; Chatterjee, G. C.; Ghosh, N. C.; Ghosh, J. J.; and Guba, B. C. (Dept. of Applied Chemistry, Calcutta University, India): BIOLOGICAL SYNTHESIS OF L-ASCORBIC ACID IN ANIMAL TISSUES: CONVERSION OF D-GLUCURONOLACTONE AND L-GULONOLACTONE INTO L-ASCORBIC ACID. *Biochem. J.* 76:279-92, August 1960.

The enzyme system catalyzing the conversion of D-glucuronolactone into L-ascorbic acid in the presence of potassium

cyanide (50 mM) has been found to be located entirely in the microsomal fractions of the liver homogenates of the rat and goat and the kidney homogenate of the chick. This conversion requires no added cofactor.

The microsomes can act only on D-glucuronolactone and not on the corresponding free acid. The soluble supernatant contains a strong lactonase hydrolyzing D-glucuronolactone to D-glucuronic acid and thus inhibiting the cyanide-stimulated microsomal conversion of D-glucuronolactone into L-ascorbic acid.

Soluble-enzyme preparations have been obtained from the microsomal fractions of the liver tissues of the rat and goat and the kidney tissue of the chick. Some properties of these enzyme preparations in the conversion of D-glucuronolactone and L-glucuronolactone into L-ascorbic acid have been studied.

J.A.

Collens, William S.; and Banowitch, Morris M. (Maimonides Hosp. and State Univ. of New York Downstate Medical Center, Brooklyn, N.Y.): USE OF ORAL HYPOLYCEMIC AGENTS IN TREATMENT OF DIABETES MELLITUS: A CRITICAL ANALYSIS. *New York J. Med.* 60:2689-701, Part I, Sept. 1, 1960.

Diabetes mellitus is a complex disease in which, among other things, there occur disturbances in the peripheral utilization of glucose and glycogenesis. Any agent used in the treatment of diabetes should be capable of reversing both of these disturbed mechanisms. The literature is reviewed and there does not appear to be any definitive evidence that the sulfonylurea compounds are capable of reversing the disturbance in carbohydrate metabolism which occurs in diabetes mellitus. These compounds, in lowering the blood sugar of the patient, appear to produce carbohydrate starvation at the peripheral cell level and actually create the same metabolic condition which severe dietary restrictions accomplished in the pre-insulin era. The oral hypoglycemic agents, including the sulfonylurea compounds and phenformin hydrochloride, fail to correct the defect in carbohydrate metabolism occurring in diabetes mellitus. The evidence that the sulfonylureas stimulate the endogenous production of insulin from the beta cell is inconclusive. The use of phenformin hydrochloride appears to indicate an effect on glycogen metabolism which is not in keeping with normal muscle function. The use of presently known oral hypoglycemic agents serves as a good tool in carbohydrate research. Their use in the treatment of the human diabetic patient, judging from the literature, seems to be premature and of dubious value. W.R.K.

Crevel, S. van (Pediatric Clinic of the University of Amsterdam, Netherlands): GLYCOGEN DISEASE. *Arch. Dis. Childhood* 34:298-301, August 1959.

Follow-up studies are reported on two previously reported (1928, 1932) cases of hepatomegalic glycogen storage disease. The patients are at present thirty-two and thirty-eight years of age and in good health. Fasting blood sugars are low normal. Administration of glucagon had no effect on blood sugar, nor did epinephrine, although the latter produced ketonuria. Glycogen and cholesterol were still increased in the blood of one patient. R.L.J.

Dobson, Harold L.; Guilak, Hoosbang; Scogin, John T.; and Montgomery, C. Hunter (Metabolic Section, Dept. of Internal Medicine, Baylor University College of Medicine and the Jefferson Davis Hosp., Houston, Tex.): PHENFORMIN (DBI)

THERAPY IN DIABETES MELLITUS. *Am. Pract. & Digest Treat.* 11:587-93, July 1960.

Phenformin was used as the sole therapeutic agent or as an adjunct to exogenous insulin in ninety-three clinic diabetic patients. The detailed results are discussed. The drug was found effective after failure or toxicity of the sulfonylurea drugs but the reverse was found unlikely. Among twenty-nine unstable or difficult-to-control diabetic patients, the control of twenty-seven was improved by the use of this drug.

Gastrointestinal side effects limited the use of the drug in 12 per cent and were present in another 23 per cent. No episodes of significant hypoglycemia were encountered in this extensive trial. S.B.B.

Dodge, Warren F.; Miller, A. Pidd; and Hooks, Laura (Houston, Tex.): OPERATION OF A SUMMER CAMP FOR CHILDREN WITH DIABETES MELLITUS. *Texas J. Med.* 56:357-62, May 1960.

An attempt is made to evaluate the experience gained from the operation of a one-week pilot summer camp for children with diabetes mellitus. It would seem advisable not to include mentally retarded children in the camp. Meal planning appears to be more convenient and still quite satisfactory when the exchange system is used. To reduce the risk of hypoglycemic reactions associated with the increased activity of camp, between-meal and bedtime snacks as well as a reduction in insulin dosage are advisable. A reduction of approximately 20 per cent from the usual home requirements would seem to suffice in most. However, it is suggested that a reduction below approximately 0.85 units of insulin per kilogram of body weight per day in males and below 1.05 units of insulin per kilogram of body weight per day in females be made with caution, lest it result in ketosis. In those subjects who have had their diabetes for less than two years, the lower "safe" limit of reduction would seem to be approximately 0.55 to 0.60 units of insulin per kilogram of body weight per day. W.R.K.

Editorial. DIABETES AND THE PITUITARY GLAND. *New England J. Med.* 263:407, Aug. 25, 1960.

The pancreas still remains the dominant organ in the etiology of diabetes but attention is now directed toward the complex relations of multiple etiologic factors. The pancreatic hormone insulin continues to be the universally effective therapeutic agent but its action depends not merely on the replacement of deficient insulin function but on other metabolic and endocrine activities. The accumulation of both experimental and clinical evidence has demonstrated clearly the potential influence of the pituitary gland. Physicians must be alert to the possibility of profound changes in the response of diabetes to insulin therapy under circumstances in which the impairment of pituitary function may occur. W.R.K.

S. K. Fineberg, M.D. (Med. Serv., Harlem Hosp., New York, N.Y.): CLINICAL EXPERIENCE WITH CHLORPROPAMIDE AND COMPARATIVE EVALUATION WITH TOLBUTAMIDE. *J. Am. Geriatrics Soc.* 8:441-48, June 1960.

A clinical study of the effect of chlorpropamide in fifty patients with "maturity-onset" diabetes is presented and compared with the effect of tolbutamide in a similar but smaller group of thirty-five patients selected by the same criteria. The findings of these studies were: (1) Chlorpropamide produced satisfactory control of diabetes in almost twice as great a

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percentage (76 versus 43 per cent) of patients than did tolbutamide; an excellent control of more than twice as great a percentage (74 versus 31 per cent); (2) the incidence of primary and secondary failures with tolbutamide therapy was notably higher than with chlorpropamide therapy; and (3) with the dosage level used, chlorpropamide (100-500 mg./day) produced as few and possibly fewer side reactions than did tolbutamide. S.S.

Fisher, R. B.; and Zachariab, F. (Dept. Biochemistry, University of Edinburgh, Scotland): DETERMINATIONS OF INSULIN-LIKE ACTIVITY OF THE A-CHAIN OF INSULIN. *Biochem. J.* 76:155, July 1960.

Insulin-like activity of the A-chain of insulin was found in the isolated perfused rat heart on the intracellular permeation of L-arabinose. J.A.

Georas, Constantine S.; Meissner, George F.; Dillon, John A.; and Calenda, Daniel G. (Dept. of Int. Med. and Dept. of Pathology, Rhode Island Hosp., Providence, R.I.): AMELIORATION OF DIABETES MELLITUS AFTER PITUITARY INFARCTION: REPORT OF THREE CASES. *New England J. Med.* 263:374-78, Aug. 25, 1960.

Three cases of hypopituitarism in diabetic patients are reported. Autopsy findings are presented in two of these. In the third patient, who is alive and well, the diagnosis was established clinically and supported by the response to replacement therapy. The clinical, biochemical, and pathological aspects of diabetes in relation to the pituitary gland are discussed and compared to the experimental Houssay phenomenon in dogs. The literature, containing a total of twenty-five cases, is briefly reviewed. W.R.K.

Haworth, J. C.; and Coodin, Fischel J. (Dept. of Paediatrics, Univ. of Manitoba and the Children's Hosp., Winnipeg, Manitoba, Canada): IDIOPATHIC SPONTANEOUS HYPOGLYCEMIA IN CHILDREN: REPORT OF SEVEN CASES AND REVIEW OF THE LITERATURE. *Pediatrics* 25:748-65, May 1960.

Seven cases of idiopathic spontaneous hypoglycemia in children are reported. A review is made of fifty-one case reports of this condition found in the literature. Although an elevation of the blood sugar to normal levels is often seen during treatment with ACTH, some patients do not respond favorably to this treatment, nor to treatment with adrenocorticosteroids. Treatment by dietary means is of little value. The etiology is discussed. Although few cases have been studied, high levels of circulating insulin-like activity in serum have been previously recorded in two patients. A normal value in one patient is recorded here. Cases may be classified as leucine-sensitive and leucine-insensitive according to the blood sugar response to this amino acid. The possibility exists that another cause may be an inability to raise the blood glucose by the release of adrenalin. The "syndrome of idiopathic hypoglycemia of infants" as described by McQuarrie is believed by the authors not to be a single clinical entity. W.R.K.

Hellerström, Claes; Petersson, Birger; and Hellman, Bo (Histological Dept., University of Uppsala, Sweden): SOME PROPERTIES OF THE B CELLS IN THE ISLETS OF LANGERHANS STUDIED WITH REGARD TO THE POSITION OF THE CELLS. *Acta endocrinol.* 34:449-56, July 1960.

In an effort to determine anatomical indices of functional activity of the beta cells, nuclear size and the position of the nucleoli within the nuclei were studied in the pancreas of the

normal rat. The smallest nuclei and the lowest number of nucleoli adjacent to the nuclear membrane were recorded in the central portions of the largest islets. These characteristics were considered to be evidence of the lower beta cell activity of these areas. This was also studied in starvation and the fed state. The mechanism of these changes was considered to be related to the position of the blood supply and the relative concentration of glucose and insulin supplied to the various areas. S.B.B.

Hellerström, Claes; Westman, Sigbild; Zachrisson, Ulla; and Hellman, Bo (Histological Dept., University of Uppsala, Sweden): THE NUMBER OF RED BLOOD CELLS IN THE ISLETS OF LANGERHANS AS AN INDEX OF THE B CELL ACTIVITY. *Acta endocrinol.* 34:611-18, August 1960.

The authors sought to test the hypothesis that the number of red blood cells in the islets of Langerhans could be used as an index of beta cell activity. However, in starved rats measurements of red blood cell content of the islets of the pancreas by a planimetric method showed an increase at the same time that their function had decreased as indicated by nuclear size. This increase was both absolute and relative to that of the exocrine portion of the pancreas. This indicates a lack of usefulness of this measurement as an indication of beta cell function. S.B.B.

Hiatt, Howard H. (Dept. of Med., Harvard Medical Sch. and the Dept. of Medical Research, Beth Israel Hosp., Boston, Mass.): CARBOHYDRATE METABOLISM IN PENTOSURIA. *Ann. Int. Med.* 53:372-79, August 1960.

C¹⁴-labeled glucuronolactone was administered alone and together with imidazole-acetic acid to patients with pentosuria. The excretion of C¹⁴-labeled CO₂ and of imaariboside was measured and compared with that of normal humans. The results confirmed the view that the prime metabolic defect in pentosuria is a block in glucuronic acid metabolism. Further studies of serum and urinary pentose levels ruled out a defect in renal excretory function as the primary defect in pentosuria. S.B.B.

Jacobsen, Dora (Inst. of Physiol., University of Lund, Sweden): EFFECTS OF THYROXINE ON GROWTH OF MAMMARY GLANDS, WHOLE BODY, HEART AND LIVER IN HYPOPHYSECTOMIZED RATS TREATED WITH INSULIN, CORTISONE AND OVARIAN STEROIDS. *Acta endocrinol.* 35:107-34, September 1960.

The author measured the growth effects of various combinations of insulin and ovarian (oestradiolbenzoate and progesterone), adrenal (cortisone acetate), and thyroid (D L-thyroxine) hormones upon gonadectomized and hypophysectomized rats. The results indicated that the metabolic effects of adding thyroid hormone to the other hormones enhanced the development of end buds in the mammary glands and of the whole body growth (in length, as contrasted with fat alone).

Thyroid and adrenal hormones were also required for survival as well as growth. S.B.B.

King, Francis P. (New Bern, N.C.): PARALYSES OF THE EXTRAOCULAR MUSCLES IN DIABETES. *A.M.A. Arch. Int. Med.* 104:318-22, July 1959.

Three cases of paralysis of the extraocular muscles in patients with diabetes mellitus are presented. In one, the sixth, and in two, the third, cranial nerves were involved. In the

youngest patient, fifty-seven years old, the initial clinical manifestation of diabetes was the ophthalmoplegia. The syndrome is characterized by weakness of the muscle innervated by one nerve. Pain in the involved eye or side of the face is usually present and often severe. Recovery of the ocular paralysis was complete within three months in each patient. This is an important feature in differential diagnosis. The pathogenesis is apparently due to an ischemia of the nerve due to a lesion of the vasa nervorum. This may be a specific angiopathy related to the retinopathy and nephropathy. B.F.K.

Korner, A. (Dept. Biochem., University of Cambridge, England): THE EFFECT OF ADMINISTRATION OF INSULIN TO THE HYPOPHYSECTOMIZED RAT ON THE INCORPORATION OF AMINO ACIDS INTO LIVER PROTEINS IN VIVO AND IN A CELL-FREE SYSTEM. *Biochem. J.* 74:471-78, March 1960.

Insulin treatment increased the incorporation into rat liver proteins of intravenous injected labeled amino acids. Hypophysectomy results in a decreased incorporation of amino acids into protein by cell-free systems; however, previous treatment of animals with insulin enhanced the ability of liver microsomes to incorporate labeled amino acids into protein. Female hypophysectomized rats respond more quickly and to a greater extent to the stimulating effects of insulin treatment than male hypophysectomized rats. J.A.

Krall, Leo P. (Joslin Clin. and New England Deaconess Hosp., Boston, Mass.): FAT: ITS RELATIONSHIP TO THE TREATMENT OF DIABETES MELLITUS. *Acad. Med. New Jersey Bull.* 6:59-63, June 1960.

In spite of the increase of information concerning the lipids in recent years, it appears that definitive evidence of their function is still incomplete. Certain facts are evident. The body fats are an important source of stored energy in the body. They also have an important function in insulin utilization. Cholesterol is the most readily measured lipid in general medical practice and its level varies as influenced by certain conditions of health and disease. Inadequately treated diabetes may produce abnormal blood levels which, in severe cases, may be associated with lipemia retinalis and xanthomatosis. Further possible relationship to atherosclerotic vascular disease may be postulated. In the earlier stages, these abnormalities may be reversible by adequate treatment. The injudicious use of oral hypoglycemic agents (or any other mode of treatment) may permit poorly controlled diabetes with consequent abnormalities of lipid metabolism. In the overweight, the total calories and fat in the diabetic diet should be low enough to allow return to an optimal weight. The present evidence suggests that massive substitution of unsaturated fats (usually lipid fats of vegetable origin) can be influential in reversing elevated blood lipid levels, but the practicality of this as well as its ultimate usefulness and desirability have yet to be determined. W.R.K.

Levy, Carroll M. (Div. of Hepatic Metabolism and Nutrition, Dept. of Med., Jersey City Med. Center and Seton Hall Coll. of Med., Jersey City, N.J.): FAT, THE LIVER AND DIABETES. *Acad. Med. New Jersey Bull.* 6:53-58, June 1960.

Diabetes mellitus is often associated with fatty liver. It may be due to nonrelated causes but is frequently secondary to metabolic disturbances inherent in the diabetic syndrome. The precise alteration promoting a disparity between rate of removal and deposition of neutral fat in the liver in these in-

stances is not known. It is of major clinical significance in that continuation of the process responsible for fatty liver may eventually lead to cirrhosis. Occasionally hepatic steatosis is associated with hyperglycemia and glycosuria which disappears with restoration of normal liver morphology. This may be due to disturbances of hepatic regulation of blood sugar or latent diabetes made manifest by liver injury. W.R.K.

Markkanen, Antti; Oka, Martti; and Peltola, Pentti (Medical Department Kivela Hosp., Helsinki): CARBUTAMIDE IN DIABETES: REPORT OF A LONG-TERM TRIAL, WITH SPECIAL REFERENCE TO LATE FAILURES. *Brit. M. J.* 1:1089-91, April 9, 1960.

This investigation was carried out to assess the value of prolonged treatment with an orally administered hypoglycemic drug, carbutamide. The series comprised 262 diabetics who had started on oral treatment twenty-eight to forty-two months earlier. The treatment was unsuccessful during the first month in 18 per cent of a group of 148 patients with diabetes previously treated by diet alone. Later in the course of the treatment there were failures in 27 per cent of the remaining cases in this group. Among these late failures were patients who were considered most suitable for this treatment.

In the group of 114 patients previously treated with insulin there were primary failures during the first month in 56 per cent and late failures in 50 per cent of the patients who at first reacted successfully. The average insulin requirement did not increase after the failure of oral therapy. J.A.

Masley, Peter M.; Bonanno, Charles A.; and Grace, William J. (Dept. of Med., St. Vincent's Hosp. of the City of New York, New York, N.Y.): DIABETES AND STEATORRHEA IN PRIMARY CARCINOMA OF THE PANCREAS. *Ann. Int. Med.* 52:1147-60, May 1960.

Two patients are reported with diabetes and steatorrhea as the initial symptoms of carcinoma of the pancreas. No others were found in an experience with sixty-three patients with carcinoma of the pancreas in three years. The pathogenesis and mechanism of these individual symptoms of carcinoma of the pancreas were discussed and the importance of considering the diagnosis in the presence of the two together emphasized. S.B.B.

Osler, Mogens (Royal Maternity Dept. B, Rigshospitalet, Copenhagen, Denmark): BODY FAT OF NEWBORN INFANTS OF DIABETIC MOTHERS. *Acta endocrinol.* 34:277-86, June 1960.

The reported overweight (by 550 gm.) of newborn infants of diabetic mothers was investigated by measurement of body fat in twelve infants born of diabetic mothers, seventeen infants born of normal mothers and eight "control" infants born of normal mothers but of the same gestational age as the first group.

The obesity was determined by caliper measurement of skin-fold thickness and by X-ray determination of subcutaneous fat thickness.

An increase of skin thickness attributable to fat was found in the newborn infants of diabetic mothers. This was 38 to 46 per cent greater than the normal and 50 per cent greater than the control group. S.B.B.

Osler, Mogens (Royal Maternity Dept. B, Rigshospitalet, and University Inst. of Biological Chemistry, Copenhagen, Denmark): BODY WATER OF NEWBORN INFANTS OF DIABETIC MOTHERS. *Acta endocrinol.* 34:261-76, June 1960.

Body water studies were conducted in twenty-three newborn

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infants of diabetic mothers and fifteen of normal mothers. The total body water was determined by a method using heavy water (deuterium oxide), water of the extracellular compartment by a dilution method using thiosulfate and intracellular water by the difference between the two.

It was concluded that the infants born of diabetic mothers were obese in view of the normal weight and a decrease in total body water. There was a more significant decrease in extracellular than intracellular water. This was interpreted as due to an increased deposition of glycogen and was attributed to hypoglycemia and increased secretion of insulin. S.B.B.

Osler, Mogens (Royal Maternity Dept. B, Rigshospitalet, Copenhagen, Denmark): NEONATAL CHANGES IN BODY COMPOSITION OF INFANTS BORN TO DIABETIC MOTHERS. *Acta endocrinol.* 34:299-304, June 1960.

Comparative studies were made of total body water in per cent of total body weight at birth after maximal weight loss (four or five days) after birth in normal infants and those born to diabetic mothers. The lack of increase or slight decrease found in the latter group was interpreted to indicate lack of extra fat catabolism during this period of semistarvation. It was attributed to excess beta cell function in this group in the immediate postpartum stage. S.B.B.

Osler, Mogens (Royal Maternity Dept. B, Rigshospitalet, Copenhagen, Denmark): RENAL FUNCTION IN NEWBORN INFANTS OF DIABETIC MOTHERS. *Acta endocrinol.* 34:287-98, June 1960.

Glomerular filtration rate, water, electrolyte and nitrogen excretion were studied in fourteen infants born to diabetic mothers, ten normal full-term infants and four premature infants during the first two to three days after birth.

Glomerular filtration rate was low immediately after birth in both premature and diabetic groups and both had high water, electrolyte and nitrogen excretion. However, the diabetic group excreted more water per unit of nitrogen excreted.

This was interpreted to indicate that both groups utilized their own body protein immediately postpartum but that the diabetic group had excess glycogen to utilize as a source of both energy and water in this period. S.B.B.

Parker, Arthur M.; and Gitzelter, Louis (Diabetic Clin., Kings County Med. Center, Brooklyn, N.Y.): THE ROUTINE USE OF TOLBUTAMIDE IN UNSELECTED PATIENTS IN A LARGE DIABETIC OUTPATIENT CLINIC. *J. Am. Geriatrics Soc.* 8:550-55, July 1960.

Experiences with the use of oral tolbutamide in a large diabetic clinic in a municipal hospital are reported involving 275 patients of various ages, extractions and states of disability. The degree of cooperation varied widely. The result showed that regardless of the lack of universal success in the control of the diabetic state, tolbutamide is relatively safe and effective and therefore has a place in difficult outpatient diabetic management. However, since the drug has been reported as not completely nontoxic and the initial response is unpredictable the patients initially should be under close observation. In this series, the over-all failure rate was 35.6 per cent. This was attributed to the fact that these patients were not strictly selected according to established criteria and were a more uncooperative group than in some other series. If there is a choice between insulin and tolbutamide therapy in the type of diabetic patient here described, tolbutamide is far more convenient for both patient and physician. S.S.

Pedersen, Jørgen; Lund, Flemming; and Ringsted, Jørgen (Med. Dept. C, Surgical Dept. F and Pathological Inst., Bispebjerg Hosp., Copenhagen, Denmark): HYPOGLYCEMIA IN THE PRESENCE OF MASSIVE FIBROSARCOMA (MESENCHYMOMA). *Acta endocrinol.* 34:148-56, May 1960.

The authors describe the occurrence of severe hypoglycemia characteristic of islet cell tumor in a seventy-one-year-old woman with a large retroperitoneal fibrosarcoma. It was located near the right renal pelvis. All symptoms disappeared after its removal. It gave histological evidence of malignancy but chemical and biological analysis did not reveal the presence of insulin. S.B.B.

Ponz, F.; Pares, R.; Planas, J.; and Lluch, M. (Laboratoire de Physiologie animale, Faculte des Sciences, Universite de Barcelone, Barcelona, Spain): SEVERAL METABOLIC ACTIONS OF GLUCAGON. *Bull. Soc. Chim. Biol. Suppl.* 4:51-57, 1957.

An action of glucagon on yeast was found. An increase in endogenous alcoholic fermentation is caused by this hormone. Hitherto no action of glucagon on cell respiration had been found. The authors noted that amorphous glucagon and inactive insulins have an inhibitory effect on the respiration of yeast cells.

Glucagon seems to have a definite effect in kidney homogenates. It seems to activate endogenous respiration probably through its glycogenolytic action. In presence of excess succinate, however, it causes a slight fall in oxygen consumption. Glucagon slightly reduces the effect of cytochrome C on succinate oxidation.

Intravenous injection of glucagon in rats produces a fall in intestinal absorption of glucose of from 0.2 mg. glucagon per animal. Subcutaneous injection has no effect. It was noted that the inhibition is unrelated to the blood glucose content. W.R.K.

Pote, William W. H., Jr. (School of Medicine, Coll. of Med. Evangelists, Los Angeles, Calif.): ORAL TREATMENT OF DIABETES. *M. Arts & Sc.* 14:56-62, Second Quarter 1960.

Dietary control is still the mainstay of treatment in over half of all persons with diabetes. Oral agents or insulin should be used only when additional need is demonstrated. Whichever treatment is used, the patient must be observed carefully to determine the adequacy of control of the diabetes. Hypoglycemic agents are still under clinical trial. Practicing physicians can provide vital information as to the practical, long-term clinical usefulness and dangers of these drugs as well as information regarding the effects on diabetic sequelae. W.R.K.

Ralli, Elaine P. (New York University-Bellevue Medical Center, New York, N.Y.): NUTRITIONAL DISTURBANCES ASSOCIATED WITH DIABETES MELLITUS. *Postgrad. Med.* 26:612-16, November 1959.

The various metabolic parameters which may be disturbed by abnormalities in diet and regulation in diabetes mellitus are discussed, including protein, fat, vitamins, and electrolytes. S.B.B.

Reinikainen, M. (Med. Dept., General Hosp., Tampere, Finland): USE OF PHENETHYLDIGUANIDE (DBI) FOR TREATMENT OF DIABETES MELLITUS. *Ann. med. int. Fenniae* 48:25-31, 1959.

The effect of phenethyldiguanide (DBI) was examined in fifteen patients who had not responded satisfactorily to sulfonylurea therapy. Nine of them (60 per cent) showed a considerable fall in the blood sugar in response to this drug. In

three of them, however, nausea and vomiting necessitated cessation of treatment. The drug was of obvious benefit in six cases (40 per cent). Side effects (nausea, vomiting, diarrhea) occurred in five cases (33 per cent). The drug had no toxic effect on the function of the liver or kidneys or on blood chemistry. W.R.K.

Rice, Carl O.; Strickler, J. H.; and Beckman, Martin (1635 Medical Arts Building, Minneapolis 2; Cowles Foundation, Yale University, New Haven): THE HYPERGLYCEMIC REACTION OF ALARM IN SURGERY. A.M.A. Arch. of Surgery 79:815-19, November 1959.

Seventeen of twenty-four patients subjected to resection of the colon or stomach exhibited a sharp rise in blood glucose to more than 200 mg. per 100 ml. in the immediate postoperative period. An intravenous infusion of fructose was started twenty-four to thirty-six hours before operation in a dosage of 120 gm. every eight hours, and was continued through the third postoperative day. The blood sugar response lasted as long as twenty-four hours after surgery and usually disappeared by the end of seventy-two hours. It was accompanied by a small amount of glycosuria which was not commensurate with the degree of hyperglycemia. Blood electrolytes tended to fall during the postoperative period. A control group having less extensive surgery failed to show such a marked elevation. Subsequent glucose tolerance tests in the experimental group were normal. The phenomenon is felt to constitute an adrenal response to trauma with increased output of glucose by the liver. It is recommended that severe postoperative hyperglycemia should be treated with insulin. A.R.C., JR.

Robin, Eugene D.; Travis, David M.; Julian, Desmond G.; and Boshell, Boris R. (Department of Med., Harvard Med. School and The Med. Clin. of the Peter Bent Brigham Hosp., Boston): METABOLIC PATTERNS DURING PHYSIOLOGIC SLEEP: I. BLOOD GLUCOSE REGULATION DURING SLEEP IN NORMAL AND DIABETIC SUBJECTS. J. Clin. Invest. 38:2229-33, December 1959.

The hourly nocturnal blood sugar values were determined in sleeping subjects, throughout the night. There were eight normals and sixteen diabetic patients. Of the latter, five were diet treated, eight treated by insulin and three by tolbutamide.

The blood sugar levels were found to be remarkably constant among the normals. However, both diet and insulin treated diabetic patients showed variations, with relatively inconsistent patterns. Three milder diabetics treated with tolbutamide showed the least variability among the diabetics.

The importance of determining blood sugar levels at night in the total assessment of the blood sugar curve of a diabetic becomes apparent from this study. S.B.B.

Rodari, T.; and Specchia, G. (Istituto di Patologia Speciale Medica e Metodologica Clinica della Università Di Pavia, Italia): APPLICATION OF THE DOUBLE INTRAVENOUS HYPERGLYCEMIC TEST IN THE STUDY OF DIABETES. Acta Endocrinol. 33:157-67, February 1960.

This is an attempt to clarify the mechanism of the intravenous glucose tolerance test utilizing the assimilation coefficient. A low dose of 0.33 gm. per kilogram was deliberately chosen as one which would not if given rapidly provoke pancreatic beta cell stimulation in the normal. It was given at the start and repeated in one hour. The glucose assimilation constant was calculated for the first and second hours and the difference between the utilization of glucose during each of

those two periods noted.

The subjects chosen were six normals, fourteen thin (juvenile type) and seventeen obese (maturity onset type) diabetic patients. The blood sugar values were greater in the second hour than the first in all three groups, but elevated above 250 only in the second and third groups. The glucose utilization coefficients were decreased in both diabetic groups. The coefficient did not change between the two one-hour periods in the normal or thin diabetics, but almost doubled in the second hour in the obese diabetics.

The theoretical implications of these findings were discussed in terms of the small dose of glucose given, the increased duration of the double test, the role of the factor of the elevated blood sugar level and the insulin potential of the pancreases in the respective groups of patients studied. S.B.B.

Rogers, Wayne R.; and Holcomb, Blair (2222 N.W. Lovejoy, Portland 10, Oreg.): LENGTHY DIABETES: CAUSES AND EFFECTS. A.M.A. Arch. Int. Med. 105:746-51, May 1960.

This is a report of a study of 114 patients who lived twenty-five years or longer with diabetes mellitus. Their lengthy survival, averaging over twenty-nine diabetic years, contrasts with the general average of sixteen diabetic years. This longevity was found attributable to the leading of a well-ordered life, including, in most instances, the careful control of the metabolic defect; to developing diabetes at a younger-than-average age; and to having a more favorable heredity. Thirty per cent of the group developed no degenerative diabetic complications. Nearly all of these are yet living, while half of those with complications have died. Eighty-two per cent of the deaths were due to cardiovascular-renal conditions, and none died from diabetic coma, cancer, or primarily from an infection. In this new era when oral glucotropic agents are tending to foster lax control by some diabetics, the present study reinforces the long-held doctrine that maintenance of as nearly normal a metabolic status as possible can prolong the diabetic person's life and increase his productivity. B.F.K.

Rook, Arthur; and Champion, R. H. (Dept. of Dermatology, Addenbrooke's Hosp., Cambridge, England): PORPHYRIA CUTANEA TARDA AND DIABETES. Brit. M. J. 1:860-61, March 19, 1960.

A case is reported of porphyria in association with diabetes mellitus. The possibility that the clinical manifestation of porphyria cutanea tarda was induced by tolbutamide is considered. The association between porphyria and diabetes is discussed. J.A.

Rowe, George G.; Maxwell, George M.; Castillo, Cesar A.; Freeman, D. J.; and Crompton, Charles W. (Dept. of Med. & Cardiopulmonary Res. Lab. of the University of Wisconsin, Madison, Wisc.): A STUDY IN MAN OF CEREBRAL BLOOD FLOW AND CEREBRAL GLUCOSE, LACTATE AND PYRUVATE METABOLISM BEFORE AND AFTER EATING. J. Clin. Invest. 38:2154-58, December 1959.

This is a study of the net cerebral utilization of blood glucose, lactate and pyruvate in the human in relation to the absolute values of these substances in the blood. The study was performed on eight patients on a general ward. After an overnight fast the subjects ate breakfast and blood values and cerebral arteriovenous differences were measured for the substances mentioned every fifteen minutes for one hour. Arteriovenous differences rose quickly (fifteen minutes) for glucose and lactate but fell even to below fasting levels while the blood levels peaked at one-half hour and continued to remain high

up to sixty minutes. This was interpreted as indicating an active transport mechanism for these substances across the brain barrier, influenced only little by blood levels.

By contrast the pyruvate arteriovenous difference was dependent on the blood level. Its blood level behaved similarly to the others but the arteriovenous difference remained high throughout the experiment. S.B.B.

Rush, Thomas; and Tupper, C. John (University of Michigan, Ann Arbor, Mich.): TWO-HOUR POSTPRANDIAL GLUCOSE DETERMINATIONS IN A PERIODIC HEALTH APPRAISAL PROGRAM. *Geriatrics* 15:630-36, September 1960.

An analysis was made of 548 patients who had been given routine two-hour postprandial tests in a periodic health examination program. This procedure revealed an incidence of diabetes mellitus of 12.6 per cent, with another 7.5 per cent of the patients classified as possibly having diabetes. The advantages of the two-hour postprandial method over random urine examination or fasting blood sugar levels as screening technics were demonstrated, and the effect of the absence of a high carbohydrate preparatory diet on the two-hour levels was investigated. The study further confirmed the decrease in tolerance for glucose with advancing age. W.R.K.

Sachse, Bernd; and Blank, Heinz (Department of Medicine, Academy of Medicine, Düsseldorf, Germany): FUNCTIONAL HYPOGLYCEMIA AND ORGANIC HYPERINSULINISM. *Deutsche med. Wchnschr.* 84:1679-82, Sept. 11, 1959.

The various types of hypoglycemia and their differential diagnoses are discussed and illustrated by case demonstrations. Particular attention is called to the fact that extrapancreatic tumors can produce the picture of hyperinsulinism. This phenomenon appears to occur more often than generally recognized. The authors describe an instance in a thirty-nine-year-old man who began to develop severe hypoglycemic attacks in 1956; at the first laparotomy no islet cell tumor was found and a partial pancreatectomy was performed. The hypoglycemic episodes persisted and a second partial pancreatectomy was done about one year later. This too failed to prevent recurrence of the attacks. Shortly afterwards an intrathoracic tumor was found radiographically. A bronchoscopic biopsy revealed a sarcoma. Pneumonectomy was attempted but was unsuccessful. The patient expired soon afterwards. Although no autopsy was performed and no biochemical studies of the tumor tissue were done, the authors assume that the bronchogenic tumor was the cause of the hypoglycemic attacks. M.G.G.

Sandberg, Herschel; Min, Byong Sok; Feinberg, Leonard; and Bellet, Samuel (Division of Cardiology, Philadelphia Gen. Hosp., Philadelphia): 131 I TRIOLEIN TOLERANCE CURVES IN PATIENTS WITH DIABETES MELLITUS: THEIR SIMILARITY TO THOSE OBSERVED IN MYOCARDIAL INFARCTION. *A.M.A. Arch. Int. Med.* 105:866-72, June 1960.

131 I triolein tolerance curves were performed on twenty-seven patients with diabetes mellitus, ranging in age from nineteen to eighty-six years. Abnormal curves, as shown by elevated whole blood activity, circulating lipoprotein activity, elevated twenty-four-hour whole blood and circulating lipoprotein activity, almost identical with a previously reported myocardial infarction group, were demonstrated in diabetics under sixty with demonstrable evidence of atherosclerotic complications. Lesser degrees of abnormality were found in diabetics under sixty with no clinically demonstrable evidence of atherosclerotic

complications. Diabetics over sixty years of age displayed 131 I tolerance curves very similar to but slightly lower than the normal controls. The duration of insulin administration had no direct correlation with an abnormal tolerance curve, but patients taking larger daily doses of insulin showed higher radioactivity in their postprandial blood samples. B.F.K.

Schrade, W.; Böble, E.; and Biegler, R. (I. Dept. of Medicine, Univ. of Frankfurt, Frankfurt a.M., Germany): ON THE POLYEN-ACID CONTENT OF THE VARIOUS BLOOD LIPID FRACTIONS IN ARTERIOSCLEROSIS AND DIABETES MELLITUS. *Klin. Wchnschr.* 37:1101-09, Nov. 1, 1959.

The sera of twenty-one normal control individuals were examined and hyperlipemic sera of thirty-one arteriosclerotic and twenty-one diabetic patients. The phospholipids were separated by dialysis from the serum lipid extracts and the cholesterinesters, free cholesterol, glycerides and unesterified fatty acids by adsorption chromatography. In the hyperlipemic sera the glycerides were increased most markedly. The various fatty acid fractions obtained after alkali-isomerization were then examined spectrophotometrically and their content of unsaturated fatty acids (polyen-acids) was estimated. The cholesterol esters contained most of the polyen-acids. About 47.1 per cent of the cholesterol-ester-acids in the normal sera were represented by linoleic acid. The polyen-acid content of the phospholipid fraction was considerably smaller and amounted to about 22.2 per cent in normal sera; the glycerides of normal sera had a low linoleic acid content of about 11.2 per cent and only very small amounts were found in the unesterified fatty acids. In the lipid fractions of the sera of the arteriosclerotic patients a somewhat smaller polyen-acid content was present. The cholesterol esters contained about 38 per cent linoleic acid and the phospholipids about 19.6 per cent. These differences appeared to be statistically significant. Likewise, a statistically significant decreased content of polyen-acids was found in the cholesterol-ester, phospholipid and glycerol fractions of the hyperlipemic sera of the diabetic patients. Since the polyen-acids tend to decrease the cholesterol esters and other serum lipid fractions, it is suggested that their decreased content in the serum lipid fractions in arteriosclerosis and diabetes mellitus is responsible for the hyperlipemia in these conditions. Two possibilities for the mechanism of this disproportion of the serum lipids are discussed. On the one hand, a primary nutritional lack of higher unsaturated fatty acids may lead to an increase of the blood lipids as a whole; on the other hand, an endogenous relative lack of polyen-acids which are necessary for fat transport may occur in cases of hyperlipemia and thus sustain or augment this condition (German). M.G.G.

Schwartz, William (Heart Station, Fall River Gen. Hosp., Fall River, Mass.): DIABETES MELLITUS, IDIOPATHIC MYOCARDIAL HYPERTROPHY AND PAROXYSMAL ATRIAL FLUTTER. *J. Am. Geriatrics Soc.* 8:472-77, June 1960.

A case is reported as one of idiopathic myocardial hypertrophy associated with diabetes mellitus, in view of persistent electrocardiographic pattern of left ventricular hypertrophy and absence of roentgenologic or other evidence of valvular, congenital or hypertensive heart disease. Routine determination of the blood sugar level is recommended in all patients having myocardial injury, as nondiabetic patients may temporarily show glycosuria due to the cardiac episode. W.R.K.

Segal, Stanton; and Foley, Joseph B. (The Clin. Endocrinol. Branch, Nat. Inst. of Arthritis and Metabolic Diseases, Nat. Inst. Health, Bethesda, Md.): THE METABOLIC FATE OF C¹⁴-LABELED PENTOSSES IN MAN. *J. Clin. Invest.* 38:407-13, February 1959.

The fate of C¹⁴-labeled *d*-ribose, *d*-xylose, *d*-arabinose and *l*-arabinose was studied in man using trace amounts in contrast to previous experiments using 5 to 20 gm. amounts. These studies confirmed the fact that the first is converted to CO₂ to a larger extent than the others while *l*-arabinose is converted to CO₂ in only trace amounts. Urinary excretion accounts for the bulk of disposal, but the product excreted is largely metabolic products of the original pentoses. The possible metabolic pathways of these sugars is discussed on the basis of the data from this and previous experiments. S.B.B.

Sharpey-Schafer, E. P.; and Taylor, P. J. (Dept. of Medicine, St. Thomas's Hosp., London, S.E.1, England): ABSENT CIRCULATORY REFLEXES IN DIABETIC NEURITIS. *Lancet* 1:559-62, March 12, 1960.

Circulatory reflexes were studied by continuous arterial records, using the Valsalva maneuver, tipping, coughing, hyperventilation, and mental arithmetic to change the stroke output of the heart. Of 337 diabetic patients seventeen had absent circulatory reflexes and fourteen a partial defect of the reflex system.

The effects of indirect heating and a sudden deep inspiration on hand blood-flow were normal, indicating that the defect lay on the afferent pathway. Of other neurological lesions, impotence in males showed the best correlation with absent circulatory reflexes. Severity and duration of the diabetic state were not factors. One patient had postural syncope, and in two others arterial pressure reached syncopal levels with more severe procedures. J.A.

Sims, Erban A. H. (Dept. of Med. Coll. of Med., University of Vermont, Burlington, Vt.): RENAL FUNCTION DURING PREGNANCY COMPLICATED BY INTERCAPILLARY GLOMERULOSCLEROSIS. SERIAL STUDIES IN A YOUNG DIABETIC. *Ann. Int. Med.* 52:693-702, March 1960.

A twenty-two-year-old pregnant female with diabetes since age four was followed with serial renal function studies, such as renal clearance and glomerular filtration rate. These were performed at 10, 16, 24 and 30 weeks of pregnancy. There was an increase in glomerular filtration rate by 50 per cent above the normal and the filtration fraction initially low became normal. Tubular function did not change. Renal biopsy at onset of pregnancy showed diffuse intercapillary glomerulosclerosis. S.B.B.

Sugar, Samuel J. N.; Thomas, Lawrence J.; and Eugenio, Teodora M. (Prince Georges Genl. Hosp., Cheverly, Md., and District of Columbia Genl. Hosp., Washington, D.C.): CHLORPROPAMIDE IN THE MANAGEMENT OF DIABETES. *A.M.A. Arch. Int. Med.* 104:360-64, September 1959.

Eighty-four patients were treated with chlorpropamide from two to eleven months. Sixty-two per cent were satisfactorily controlled. The most readily controlled were middle-aged patients with relatively recent onset of diabetes who required less than 40 units of insulin daily. The unstable diabetics of any age were poor candidates for control. Of patients who lost responsiveness to tolbutamide, 62 per cent were satisfactorily managed with chlorpropamide. Toxicity was somewhat related to dosage, especially if it was more than 1.0 gm. a day. Gastrointestinal symptoms, chest pain, and muscular weakness were the most prominent side effects. B.F.K.

Wagner, David H. (Depts. of Surgery, Michael Reese Hosp., Cook County Hosp., and Chicago Med. Sch., Chicago): THE PREPARATION AND CARE OF DIABETIC PATIENTS REQUIRING SURGERY. *S. Clin. North America* 39:161-70, February 1959.

The mortality rate in operating upon diabetic patients has been reduced markedly since 1923 from 11.5 to 2.5 per cent. Depot insulin is given in half the usual dose on the day of surgery, and Crystalline Insulin is added at four-hour intervals. Energetic treatment is used for emergency procedures in severe diabetics. Serum potassium is estimated frequently and potassium added to the intravenous fluids in amounts of 20 or 40 mEq. Diabetics should receive a short-acting anesthetic, such as procaine locally or intrathecally. Cyclopropane is more desirable than ether. Postoperatively, fluid and carbohydrate balance must be followed closely. A.R.C., JR.

Wilson, Jean D.; and Siperstein, Marvin D. (Dept. of Internal Med., The Univ. of Texas Southwestern Med. School, Dallas): STUDIES ON THE RELATIONSHIP BETWEEN GLUCOSE OXIDATION AND INTERMEDIARY METABOLISM. III. THE INFLUENCE OF PYRIDINE NUCLEOTIDES ON PROTEIN SYNTHESIS. *J. Clin. Invest.* 38:317-24, February 1959.

This is an attempt to further knowledge regarding the protein sparing effect of glucose oxidation. This was performed in vitro on liver homogenates of the normal rat. The addition alone of glucose-6-phosphate, as an enhancer of ATP generation, increased both C¹⁴-labeled acetate and amino acid (dl-valine) incorporation into liver protein. It was then shown that when this ATP effect was maximal, diphosphopyridine (DPN) or triphosphopyridine (TPN) nucleotides could further enhance (double) the effect. The latter (TPN) was more consistently effective. Thus the pyridine nucleotides were shown to be potent cofactors in protein synthesis. The authors felt that the point of enhancement of protein synthesis might be at the site of ammonia fixation by alpha-ketoglutaric acid. The relationship of the two pyridine nucleotides to the respective Embden-Meyerhof pathway and hexosemonophosphate shunt was underlined as a means of correlating these in vitro effects with the in vivo protein sparing effects of normal glucose oxidation. S.B.B.

Wright, Peter H. (Guy's Hosp. Medical Sch., London, S.E.1, England): PLASMA-INSULIN ACTIVITY IN ACROMEGALY AND SPONTANEOUS HYPOGLYCAEMIA. *Lancet* 1:951-54, April 30, 1960.

Insulin assays were carried out on plasma drawn from eighteen normal subjects, fourteen acromegalics, seven patients with islet-cell tumors of the pancreas, and three children with idiopathic spontaneous hypoglycemia. Normal undiluted plasma has a biological effect equivalent to that of insulin in a concentration of approximately 70 μ U. per ml. The insulin equivalence of plasma diluted at least fourfold is about 200 μ U. per ml.

Plasma from female acromegalics, assayed in the undiluted and diluted form, has essentially normal biological activity, while at all levels of dilution plasma from male acromegalics is significantly more active. It is suggested that the latter contains high concentrations of an insulin inhibitor. Plasma from three of the cases of islet-cell tumors was abnormally active. It is concluded that normal levels of activity in suspected cases do not contraindicate this diagnosis. Normal levels of activity were found in the three cases of spontaneous hypoglycemia of infancy. J.A.

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Ninth Postgraduate Course in Diabetes and Basic Metabolic Problems

New Orleans, Louisiana, Jan. 18-20, 1961

The American Diabetes Association will hold its Ninth Postgraduate Course in New Orleans, Louisiana, Jan. 18, 19 and 20, 1961. Open to Doctors of Medicine, the Course was developed by the Committee on Professional Education of the American Diabetes Association.

Thaddeus S. Danowski, M.D., Pittsburgh, is Chairman of the Committee and Director of the Course. Arthur R. Colwell, Sr., M.D., Chicago, is Vice Chairman of the Committee. The site of the Scientific Program will be the Louisiana State University School of Medicine, and the Jung Hotel will serve as headquarters.

Daniel W. Hayes, M.D., Louisiana State University, Governor of the American Diabetes Association for the State of Louisiana, is Chairman of the Local Committee, whose members include: Joseph F. Dingman, M.D., Tulane University; A. Seldon Mann, M.D., Tulane University; Sol B. Stern, M.D., Tulane University;

C. Y. Bowers, M.D., Louisiana State University; and M. David Payne, M.D., Louisiana Academy of General Practice. First day of the Course, Wednesday, January 18, on "Clinical Aspects of Lipids," is being held in cooperation with the Metabolism Study Section of the National Institutes of Health, which will meet in New Orleans January 14-17.

REGISTRATION: A Preliminary Program which includes an application form is being mailed to all members of the Association and to subscribers of DIABETES. This form should be filled out and mailed together with the fee as soon as possible to the American Diabetes Association, Inc., 1 East 45th Street, New York 17, N. Y. Applications will be accepted in the order received and registrations officially confirmed. The registration desk will be open at the Jung Hotel, Tuesday, January 17, from 3:00 to 9:00 p.m., and at the Louisiana

ORGANIZATION SECTION

State University Auditorium, Wednesday morning at 8:00 a.m.

FEEs: The registration fee for the three-day Course is \$40 for members of the American Diabetes Association, and \$75 for nonmembers. The full fee must be paid at the time of filing application, and includes the cost of the Banquet to be held Wednesday evening, January 18. The fee will be returnable by the Association to any registrant who submits his withdrawal in writing no later than Jan. 6, 1961.

Fellows, residents, graduate students and interns in medicine and allied sciences in schools and hospitals in the New Orleans

area, may attend the Scientific Sessions without charge. They should register by January 6. Medical students in the New Orleans area may register without charge after January 6, if space permits.

HOTEL ACCOMMODATIONS: The Jung Hotel will serve as the headquarters hotel. Reservation cards will be sent to registrants.

POSTGRADUATE CREDIT: The American Academy of General Practice will give seventeen hours' Postgraduate Credit for the Course (Category II).

PRELIMINARY PROGRAM

WEDNESDAY MORNING, JANUARY 18

- 9:00 Welcome by Franklin B. Peck, Sr., President
CLINICAL ASPECTS OF LIPIDS
- 9:10 Vascular Lesions in the Diabetic—Irrving Graef
- 9:40 The Synthesis of Tissue Fat, Carbohydrate and Protein—Marvin D. Siperstein
- 10:20 Question and Discussion Period
- 10:30 *Intermission*
- 10:45 Effects of Hormones on Tissue Fats—Albert E. Renold
- 11:25 Agents which Modify Synthesis, Transport or Fate of Cholesterol—David Kritchevsky
- 12:05 Question and Discussion Period

WEDNESDAY AFTERNOON, JANUARY 18

- 2:30 Dietary Effects on Human Fat Metabolism—Edward H. Ahrens, Jr.
- 3:10 Endocrine Aspects and Hormone Therapy—T. S. Danowski
- 3:50 Question and Discussion Period
- 4:00 *Intermission*
- 4:15 Clinical Status of the Problem of Vascular Disease in Diabetics and Nondiabetics—Campbell Moses
- 4:55 Question and Discussion Period

WEDNESDAY EVENING, JANUARY 18

- 6:30 Social Hour (by subscription)
- 7:30 Banquet

THURSDAY MORNING, JANUARY 19

- 9:00 Enzyme Actions in Carbohydrate Metabolism—James Ashmore
- 9:25 Carbohydrate, Fat and Protein Metabolism in Liver and Muscles—Rachmiel Levine
- 9:50 Regulation of Insulin Secretion and Effects—Arnold Lazarow
- 10:15 Intermediary Metabolism in Diabetes and Other Endocrinopathies—Laurance W. Kinsell
- 10:40 *Intermission*
- 10:55 A Concept of the Diabetogenic Period in Man with Study of One Parameter—Jerome W. Conn
- 11:20 **PANEL: DIABETES AS A BIOLOGICAL SPECTRUM: PATHOGENESIS AND MANIFESTATIONS**—James Ashmore, Rachmiel Levine, Arnold Lazarow, Laurance W. Kinsell, Jerome W. Conn

THURSDAY AFTERNOON, JANUARY 19

SPECIAL PROBLEMS IN CLINICAL DIABETES

- 2:00 Reproduction in Diabetes—Joseph H. Crampton
- 2:20 New and Long-standing Juvenile Diabetes—Harvey C. Knowles, Jr.
- 2:40 Diabetes as an Added Problem in Medical and Surgical Practice—A. Seldon Mann
- 3:00 Infections of the Genitourinary Tract in Diabetes—Laurentius O. Underdahl
- 3:20 Why Is Diabetic Acidosis Still Fatal?—T. S. Danowski
- 3:40 *Intermission*
- 4:00 **SEMINARS ON CURRENT PROBLEMS IN DIABETES**

Each member of the Faculty for this day will serve as the leader for a small seminar. The title of each lecturer's paper will serve as the subject for his discussion group.

FRIDAY MORNING, JANUARY 20

- 9:00 The Interpretation of Laboratory Tests—Francis D. W. Lukens
- 9:20 Diet, Insulin and Oral Hypoglycemic Agents: Comparisons and Contrasts—Blair Holcomb
- 9:40 Is There a Place to Combine Insulin and Oral Hypoglycemic Therapy?—E. Paul Sheridan
- 10:00 Resume of Experience with Phenformin (DBI)—Alexander Marble
- 10:20 *Intermission*
- 10:35 **PANEL: TOOLS FOR THE REGULATION OF DIABETES MELLITUS**—Francis D. W. Lukens, Blair Holcomb, E. Paul Sheridan, Alexander Marble
- 12:00 Members of the Faculty and Registrants of the Course are cordially invited to attend the Dean's Clinic at 12 noon for L.S.U. and Tulane University junior and senior medical students, and members of the intern and resident staff of Charity Hospital.

FRIDAY AFTERNOON, JANUARY 20

SYNDROMES RELATED TO DIABETES MELLITUS

- 2:15 Hypoglycemias—Kelly M. West
- 2:35 Angiopathies—George J. Hamwi
- 2:55 Nephropathies—Victor E. Pollak
- 3:20 Neuropathies—Richard M. Paddison
- 3:40 *Intermission*
- 4:00 **CLINICAL PATHOLOGICAL CONFERENCE**

ORGANIZATION SECTION

FACULTY OF THE NINTH POSTGRADUATE COURSE

EDWARD H. AHRENS, JR., M.D., *Professor and Member, The Rockefeller Institute; Physician, The Rockefeller Institute Hospital, New York, New York.*

JAMES ASHMORE, PH.D., *Chairman and Professor, Department of Pharmacology, Indiana University Medical Center, Indianapolis, Indiana.*

JEROME W. CONN, M.D., *Professor of Medicine and Director, Metabolism and Research Unit, University of Michigan Medical School; Chief, Department of Endocrinology and Metabolism, University Hospital, Ann Arbor, Michigan.*

JOSEPH H. CRAMPTON, M.D., *Clinical Professor of Medicine, University of Washington School of Medicine; Chief, Section on Metabolism and Endocrinology, The Mason Clinic, Seattle, Washington.*

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IRVING GRAEF, M.D., *Associate Professor of Clinical Medicine, New York University Postgraduate Medical School; Visiting Physician, Bellevue Hospital; Attending Physician and Chief, Metabolic Clinic, Lenox Hill Hospital, New York, New York.*

GEORGE J. HAMWI, M.D., *Associate Professor of Medicine, Head, Division of Endocrinology and Metabolism, Ohio State University College of Medicine; Head, Section of Endocrinology and Metabolism, University Hospital, Columbus, Ohio.*

BLAIR HOLCOMB, M.D., *Clinical Professor of Medicine, University of Oregon Medical School; Staff Physician, Good Samaritan Hospital, Portland, Oregon.*

LAURANCE W. KINSELL, M.D., *Director, Institute for Metabolic Research, Alameda County Medical Institutions, Oakland, California.*

HARVEY C. KNOWLES, JR., M.D., *Associate Professor of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.*

DAVID KRITCHEVSKY, PH.D., *Associate Member, Wistar Institute of Anatomy and Biology; Assistant Professor of Biochemistry in Medicine, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.*

ARNOLD LAZAROW, M.D., PH.D., *Professor and Head, Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota.*

RACHMIEL LEVINE, M.D., *Chairman and Professor, Department of Medicine, New York Medical College; Director, Medical Services, Flower and Metropolitan Hospitals, New York, New York.*

FRANCIS D. W. LUKENS, M.D., *Professor of Medicine and Director, George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania.*

A. SELDON MANN, M.D., *Associate Professor of Clinical Medicine, School of Medicine, Tulane University; Head of Medical Section, Ochsner Clinic and Ochsner Foundation Hospital; Visit-*

ing Physician, Charity Hospital; Consultant, Crippled Children's Hospital, New Orleans, Louisiana.

ALEXANDER MARBLE, M.D., *Assistant Clinical Professor of Medicine, Harvard Medical School; Joslin Clinic and New England Deaconess Hospital, Boston, Massachusetts.*

CAMPBELL MOSES, M.D., *Director, Addison H. Gibson Laboratory, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.*

RICHARD M. PADDISON, M.D., *Professor of Neurology, School of Medicine, Louisiana State University; Chief, Louisiana State University Neurology Service, Charity Hospital, New Orleans, Louisiana.*

VICTOR E. POLLAK, M.D., *Assistant Research Professor of Medicine, Department of Medicine, University of Illinois College of Medicine; Attending Physician, Research and Educational Hospital; Adjunct Physician, Presbyterian-St. Luke's Hospital, Chicago, Illinois.*

ALBERT E. RENOLD, M.D., *Assistant Professor of Medicine, Harvard Medical School; Director, Baker Clinic Research Laboratory, Boston, Massachusetts.*

E. PAUL SHERIDAN, M.D., *Assistant Clinical Professor of Medicine, University of Colorado Medical Center; Active Staff, St. Luke's and Presbyterian Hospitals; Consulting Staff, Children's Hospital, Denver, Colorado.*

MARVIN D. SIPERSTEIN, M.D., *Associate Professor of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas.*

LAURENTIUS O. UNDERDAHL, M.D., *Assistant Professor of Medicine, Mayo Foundation, Graduate School, University of Minnesota; Consulting Physician, Section of Medicine, Mayo Clinic, Rochester, Minnesota.*

KELLY M. WEST, M.D., *Assistant Professor of Medicine, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.*

21st ANNUAL MEETING

New York City will be host to the American Diabetes Association at the Twenty-first Annual Meeting, to be held at the Hotel Commodore June 24-25, 1961, immediately prior to the annual session of the American Medical Association. Additional information about the Annual Meeting, together with a hotel reservation form, has been mailed to members of the Association.

SCIENTIFIC PROGRAM

Physicians and other scientists who would like to present papers at the Scientific Sessions are invited to submit abstracts to Jerome W. Conn, M.D., Chairman of the Committee on Scientific Programs. Seven copies of the abstracts, which are requested in order to expedite review by the Committee, should be sent as soon as possible, but not later than March 1, 1961, to Dr. Conn in care of the national office.

As previously announced, the Twenty-second Annual Meeting will be held June 9-10, 1962, at the Conrad Hilton Hotel, Chicago.

ORGANIZATION SECTION

BOARD OF STATE GOVERNORS

New officers were elected to the Board of State Governors on June 10, 1960, at the time of the Twentieth Annual Meeting. Joseph L. Izzo, M.D., Rochester, New York, was re-elected Chairman. By reason of this office Dr. Izzo will continue to serve as an ex officio member of the Council of the American Diabetes Association.

Robert C. Hardin, M.D., of Iowa City, Iowa, was elected Vice Chairman, and Eleanor A. Waskow, M.D., of Phoenix, Arizona, was elected Secretary of the Board.

Governors who will serve for the organizational year 1960-61 are:

State	Term Expiring June 1963	Governor
Alabama		Samuel Eichold
Arizona		Eleanor A. Waskow
Arkansas		Hal Dildy
California		
Northern & Nevada		Mervyn U. Schwartz
Southern		Roy F. Perkins
Colorado		W. Bernard Yegge
Connecticut		Barnett Greenhouse
Delaware		Lewis B. Flinn
District of Columbia		Maurice Protas
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Georgia		Christopher J. McLoughlin
Hawaii		Coolidge S. Wakai
Idaho		Glenn Q. Voyles
Illinois		
Northern		James B. Hurd
Southern		Thomas D. Masters
Indiana		William R. Kirtley
Iowa		Robert C. Hardin
	Term Expiring June 1961	
Kansas		Eldon S. Miller

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Louisiana	Daniel W. Hayes
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Missouri	Henry E. Oppenheimer
Montana	F. Hughes Crago
Nebraska	Morris Margolin
Nevada	See Northern California
New Hampshire	Jackson W. Wright
New Jersey	Benjamin Saslow
New York	
Eastern	Alfred E. Fischer
Western	Joseph L. Izzo
North Carolina	William M. Nicholson
North Dakota	Edgar A. Haunz

	Term Expiring June 1962
Ohio	Cecil Striker
Oklahoma	Robert C. Lawson
Oregon	Wm. Richey Miller
Pennsylvania	
Eastern	Frederick G. Helwig
Western	L. Lewis Pennock
Rhode Island	Louis I. Kramer
South Carolina	Robert Wilson
South Dakota	Everett W. Sanderson
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Texas	Hugo T. Engelhardt
Utah	William H. Bennion
Vermont	George W. Welsh, III
Virginia	William R. Jordan
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 ward Tolstoi

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Henry E. Marks, Herbert Pollack, J. Richard Connelly

DECEMBER 1 DEADLINE FOR 1961 LILLY AWARD

Nominations for the fifth Lilly Award, which will be given at the Twenty-first Annual Meeting, June 24-25, 1961, in New York City, must be received on or before Dec. 1, 1960. This annual award is supported by Eli Lilly and Company and consists of \$1,000 and a medal. The following stipulations govern the contest.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before December 1 of the year preceding the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to December 1 of the year preceding the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should

ORGANIZATION SECTION

decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

1960-61 GRADUATE AND MEDICAL STUDENT-INTERN ESSAY CONTESTS

Medical students, interns, and physicians within two years after their graduation from medical school, and graduate students in the basic sciences are invited to enter the ninth Graduate and Medical Student-Intern Essay Contests. Members of the American Diabetes Association and subscribers to *DIABETES* are asked to encourage participation in these contests.

\$250 Prize—for the best paper reporting original work, whether laboratory investigation or clinical observation.

\$100 Prize—for the best review article or case report.

The prize-winners as well as those receiving honorable mention also are given a one-year's subscription to *DIABETES: The Journal of the American Diabetes Association*.

The papers will be judged on the basis of the value of the material and method of presentation. Any subject relating to diabetes and basic metabolic problems may be selected.

Papers entered in the Contests should not have been previously published. Appropriate manuscripts will be considered for publication in *DIABETES*.

Contestants should submit the original and two copies of their papers. Typewritten and double-spaced, all manuscripts should be mailed with a letter of transmittal by March 15, 1961, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th Street, New York 17, N.Y.

REDUCED PRICE FOR DIABETES GUIDE BOOK

Medical Students, Interns and Residents may purchase copies of the *Diabetes Guide Book for the Physician* (Second Edition) at 50¢ per copy, or half the regular price. This reduction was approved by the Council at the Annual Meeting in Miami Beach to encourage students to utilize the Guide Book.

The Second Edition, including the special addendum on the uses of tolbutamide, follows the pattern set by the first (published in 1950) with additions and changes designed to aid the practicing physician in the management of diabetic patients. The edition was prepared by the Committee on Revision of *Diabetes Guide Book for the Physician* of the American Diabetes Association.

NEW MEMBERS

The following were elected as of Nov. 1, 1960:

Active

California

Bailey, Richard E.
Tyler, Russell D.

San Francisco
Whittier

Colorado

Unfug, Harry V.

Ft. Collins

Maryland

Chase, Henry V.

Frederick

New Jersey

Masley, Peter M.

Clifton

New York

Krutz, Herbert V.

Nyack

Sheusi, Carl J.

Niagara Falls

Other Countries

Mexico

Rivadeneira, Joaquin

Mexico City

Scotland

Duncan, Leslie J. P.

Edinburgh

NEWS OF AFFILIATE ASSOCIATIONS

The DELAWARE VALLEY DIABETES ASSOCIATION, formerly the Philadelphia Metabolic Association, held an Open Forum on Diabetes at 8:15 p.m., Monday evening, Nov. 14, 1960, during Diabetes Week, at the auditorium of the Philadelphia County Medical Society, 21st and Spruce Streets, Philadelphia. Alexander Marble, M.D., Boston, presented a paper entitled "Recent Progress in Diabetes," followed by a "Question and Answer Period" with George P. Rouse, Jr., M.D., Philadelphia, as moderator. In conclusion there was a presentation of "Menus and Recipes for Diabetics," by the Philadelphia Dietetic Association.

The DIABETES ASSOCIATION OF THE CINCINNATI AREA, in commemoration of its Twenty-fifth Anniversary, will present a "Symposium on Diabetes" on Thursday, November 17, at the Good Samaritan Hospital. The American Academy of General Practice will allow hour for hour Category II credit for this symposium. The following program will be presented:

Morning Session, Elmer A. Schlueter, M.D., Cincinnati, Chairman: "Greetings," Margaret F. Kessler, M.D., Cincinnati, President; "History of the Diabetes Association," Cecil Striker, M.D., Cincinnati, first President of the Diabetes Association and first President of the American Diabetes Association; "The Physiology of Diabetes," Thaddeus Danowski, M.D., Pittsburgh; "The Incidence and Development of Diabetes," by Harvey C. Knowles, Jr., M.D., Cincinnati; "The Child Diabetic," by George M. Guest, M.D., Cincinnati; "The Adolescent Diabetic," by Dr. Kessler; "The Fat Diabetic," by Dr. Striker.

Afternoon Session, Dr. Kessler, Chairman: "The Coronary Arteries," Noble O. Fowler, M.D., Cincinnati; "The Peripheral Arteries," John J. Cranley, M.D., Cincinnati; "The Eyes," Albert A. Brust, M.D., Cincinnati; "The Kidneys," E. Gordon Margolin, M.D., Cincinnati; "The Peripheral Nerves," J. Park Biehler, M.D., Cincinnati; "The Use of Antiglucose Agents," by Franklin B. Peck, Sr., M.D., Indianapolis; "The Treatment of Diabetes Acidosis," by Emile E. Werk, Jr., M.D., Cincinnati; "The Management of Pregnancy in Diabetes," by Stanley T. Garber, M.D., Cincinnati; "Summations and Questions," by Dr. Striker.

The Evening Session will include a social hour, followed by a dinner at which Dr. Schlueter will preside. After a welcome by Dr. Kessler, Franklin B. Peck, Sr., M.D., President of the American Diabetes Association, will speak on "Diabetes Abroad."

The INDIANAPOLIS DIABETES ASSOCIATION will hold a dinner meeting in the Athenaeum on November 17. Clifford Gastineau, M.D., Rochester, Minnesota, will speak on "Unstable Diabetics."

NEWS NOTES

DIABETES FOR THE PRACTITIONER

An intensive "Workshop on Diabetes" patterned for the practitioner is planned for Dec. 6, 7 and 8, 1960, at the Medical College of Georgia, in Augusta. The visiting faculty will include Alexander Marble, M.D., of Harvard University, and John Owen, M.D., of the University of Virginia. The course is acceptable for eighteen hours' credit by the American Academy of General Practice. Registration is limited to a small group for close participant-faculty communication. Registration fee is \$50.00 for the course. Those wishing to apply may write to Claude-Starr Wright, M.D., Director, Department of Continuing Education, Medical College of Georgia, Augusta, Georgia.

ITALIAN SYMPOSIUM ON DIABETES

The Second National Symposium on Diabetes will take place in Catania, Italy, Feb. 16-17, 1961, under the auspices of the Societa Italiana del Ricambio (Italian Society for Circulatory Diseases).

The following subjects will be discussed: "Dysfunction of the Sugar Metabolism and Hepatic Physiopathology," "Insulinemia in the Various Clinical Forms of the Diabetic Syndrome," and "Therapy Problems in Diabetes Mellitus."

Information may be secured from the Secretariat of the Second National Symposium on Diabetes, Institute of Medical Pathology, Garibaldi Hospital, Catania, Italy.

Following the two days of the Symposium, the Tenth National Congress of the Italian Society for Gerontology and Geriatrics will take place in Catania, on Feb. 18-19, 1961. On the afternoon of the first day of this meeting, a session will be devoted to the problem of assistance to aged diabetics.

PERSONALS

CHARLES H. BEST, M.D., Toronto, M. CAMPAGNOLI, M.D., Buenos Aires, GRACE A. GOLDSMITH, M.D., New Orleans, W. STANLEY HARTROFT, M.D., St. Louis, LAURANCE W. KINSELL, M.D., Oakland, HERBERT POLLACK, M.D., New York, S. VALIENTE, M.D., Santiago, T. B. VAN ITALLIE, M.D., New York, J. W. VESTER, M.D., Pittsburgh, and MICHAEL G. WOHL, M.D., Philadelphia, are members of the American Diabetes Association who participated in the program of the Fifth International Congress on Nutrition, which was held September 1-7 in Washington, D.C. Their contributions to the program follow: *Dr. Best*, member of the Organizing Committee and co-chairman of the program on lipids; *Dr. Campagnoli*, co-author of "Studies of the Absorption and Storage of Vitamin A Using a Fluorescence Technique"; *Dr. Goldsmith*, member of the Organizing Committee and the Program Committee, chairman of "Panel 1—Evaluation of Nutritional Status in Man" and co-author of "Analysis of Cholesterol- and Methyl Esters by Chromatography on Coated Glass Paper"; *Dr. Hartroft*, "Pathology of Lipid Disorders: Liver and Cardiovascular System"; *Dr. Kinsell*, co-

chairman of the Saturday afternoon program entitled "Lipids: Man—II," and co-author of "Fatty Acid Metabolism in Normal and Abnormal Human Subjects"; *Dr. Pollack*, co-chairman of the session on carbohydrates; *Dr. Valiente*, co-author of "Valor Nutritivo de la Dieta de 800 Embarazadas Chilenas y su Relacion con el Peso y la Talla de los Niños"; *Dr. Van Itallie*, co-author of "Experimentally Induced Steatorrhea in Man"; *Dr. Vester*, co-author of "Effect of Low Protein Diets on Serum Lipids in Man"; *Dr. Wohl*, co-author of "Pyridoxine Deficiency in Hyperthyroidism."

EDWARD L. BORTZ, M.D., Philadelphia, was moderator of an open forum on "Physical Fitness in Health and Disease" at the International Research Conference on "Muscle as a Tissue" at The Lankenau Hospital, Philadelphia, at the Friday evening session of the two-day meeting held November 3-4. The meeting was sponsored by the American Geriatrics Society, of which Dr. Bortz is President.

IRVING H. LEOPOLD, M.D., Baltimore, has been appointed Professor of Ophthalmology in the Johns Hopkins University School of Medicine. He will assume his new position Jan. 1, 1961. Dr. Leopold is at present Chairman, Department of Ophthalmology, The Graduate School of Medicine, University of Pennsylvania, Philadelphia. He is consultant to the National Institutes of Health, the American Foundation for the Blind, National Multiple Sclerosis Society, and the National Council to Combat Blindness. A member of the Editorial Board of DIABETES, Dr. Leopold is the recipient of five national awards for his work in ophthalmology, the most recent being the 1960 Friedenwald Medal given by the Association for Research in Ophthalmology. At the Twentieth Annual Meeting of the American Diabetes Association, he presented a summary of the Symposium on Retinopathy sponsored by the National Institute of Neurological Diseases and Blindness, which was held at Haddonfield, New Jersey, May 1-2.

EDWARD TOLSTOI, M.D., New York, will be chairman of the Committee on Laity Lectures at the Twenty-sixth Series of The New York Academy of Medicine's Lectures to the Laity, entitled "Current Issues in Medicine," which will be held November 16, December 7, Jan. 11, 1961, January 25, February 8, and March 1. He will discuss "Solving the Riddle of Diabetes" at the Wednesday, December 7, session, and will be Presiding Chairman for "The Family in Crisis," on Wednesday, November 16.

JAY TEPPERMAN, M.D., Syracuse, will present "The Nature, Qualities, and Varieties of Medical Research" on Wednesday, February 8, with IRVING GRAEF, M.D., New York, as Presiding Chairman.

The Laity Lectures begin at 8:30 p.m. and are broadcast by WBAI-FM (99.5 megacycles, FM).

NECROLOGY

JOHN S. MCARDLE, Minot, North Dakota, born September 16, 1916.

GROVER CLEVELAND PENBERTHY, Detroit, Michigan, born March 1, 1886.

SILVESTRO SILVESTRI, Rome, Italy, born 1883.

JOHN HENRY TRESCHER, Baltimore, Maryland, born December 26, 1898.

